MEASUREMENT OF ZOOPLANKTON BIOMASS BY CARBON ANALYSIS FOR APPLICATION IN SOUND SCATTERING MODELS

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NAVAL POSTGRADUATE SCHOOL
Monterey, California

THESIS

MEASUREMENT OF ZOOPLANKTON BIOMASS
BY CARBON ANALYSIS
FOR APPLICATION IN SOUND SCATTERING MODELS

by

James Carlton Radney

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by

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B.S., United States Naval Academy, 1973

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I. INTRODUCTION

The sea contains within its volume inhomogeneities of many different kinds, ranging in size from microscopic particles to schools of fish. "These inhomogeneities form discontinuities in the physical properties of the medium and thereby intercept and reradiate a portion of the acoustic energy incident upon them. This reradiation of sound is called scattering and the sum of the scattering contributions from all scatters is reverberation. It is heard as a long, slowly decaying, quivering tonal blast following the ping of an active sonar system and is often the primary limitation on system performance" (Urick, 1967).

In recent years it has become clear that volume reverberation or sound scattering strength (see Appendix A for explanation of these terms) is associated with variations in populations of marine organisms whose density and distributions are closely dependent on the zooplankton and other links in the food chain.

A. BACKGROUND

Traganza and Stewart (1973) accumulated a data base of volume reverberation measurements at 3.5 kHz for the development of a prototype model for forecasting operationally useful information to the fleet. Through the use of a regression equation, volume reverberation coverage was extended to most of the Northern Hemisphere on the basis of historical zooplankton data.

"There are a number of obvious reasons why there may be disagreement between current predicted volume reverberation from the zooplankton model and observed volume reverberation. Some can be attributed to

questionable uncalibrated scattering strength observations, poor zooplankton estimates, the lack of a sufficient number of observations to make significant regression analyses for all oceans, a need for a better understanding of food chain dynamics, and a better delineation of hydrographic, acoustic, and biological provinces" (Traganza and Stewart, 1973). It is the intent of this study to more accurately define zooplankton estimates of biomass for possible effective acoustic modeling.

B. OBJECTIVE

The objective of this thesis has been to evaluate carbon analysis for the chemical measurement of zooplankton biomass. The three phases of this research which were accomplished to make this evaluation included the following:

(1) An investigation of the capabilities of the LECO* Carbon Analyzer, which employs high-temperature dry combustion and a thermal conductivity sensor, to measure carbon in benzoic acid and casein was conducted. The biomass measurement using carbon analysis was tested by the determination of carbon content in a single marine copepod species, *Tigriopus californicus*.

(2) An estimate of the ATP (adenosine triphosphate) - carbon to total carbon ratio in this species was also determined in a joint ATP and carbon experiment with Dr. Traganza. This ratio was later employed to determine the living biomass of field net samples.

(3) Estimates of total, living, and dead biomass of oceanic populations were made from three cruises in the Monterey upwelling area. In short, a rapid (70 seconds on dry samples) and accurate (±3%) total particulate carbon analysis was developed and combined with ATP measurements for the determination of total, living, and dead zooplankton biomass.

*Laboratory Equipment Corporation

C. CARBON AS A MEASUREMENT OF ZOOPLANKTON BIOMASS

Many researchers, *e.g.* Cushing (1959), Beers and Stewart (1970), and Mullin (1969), have been concerned with the distribution of the standing crop of zooplankton over characteristic regions of the ocean. The preferred measure of zooplankton biomass is the total amount of zooplankton under a unit area of sea surface expressed in terms of organic content or weight of dry organic substances (*e.g.* mg carbon/m^2). The standing stock of plankton samples is also reported as the amount of zooplankton in a unit volume of water. "For various reasons, zooplankton sampling is presently inadequate to obtain a meaningful measure of zooplankton biomass as defined above. Various measures of amount are currently used, including displacement volume, wet weight, ash-free dry weight, dry weight and weight of dry organic matter."(National Academy of Sciences, 1969).

There is some prospect that it may be possible to estimate zooplankton biomass and production using chemical means. Sutcliffe (1965) has used deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) determinations for this purpose with some success. Also, possibilities do exist for the measurement of zooplankton biomass using the phytoplankton adenosine triphosphate (ATP) method of Holm-Hansen and Booth (1966). Some enzyme determinations have already been utilized for the determination of biomass (Aleem, 1955).

The measures of zooplankton most commonly used in the past have been displacement volume and wet weight (Nakai and Honjo, 1962). The displacement volume is determined on a drained plankton sample. It is not a measure of zooplankton alone but includes the interstitial liquid held between and by the bodies of planktonic organisms. Interstitial

liquid commonly accounts for 30 to 40 percent of volume of the sample. Wet weight determinations are made on plankton after blotting. Most of the interstitial liquid will have been removed before weighing, but "over-drying" of the plankton should be avoided. Zooplankters can differ markedly in the amount of organic constituents in their bodies per unit volume (water plankters such as salps compared with crustacean plankters such as copepods). The only consistent measure of biomass appears to be the amount of dry organic matter per unit volume of water sampled (National Academy of Sciences, 1969). Such analyses have been published by Krey (1958) and Curl (1962).

Previous approximations of biomass have been calculated in terms of organic content by Beers and Stewart (1970) for various size groups of microzooplankton (20-200 μm). From the volume estimates assuming a specific gravity of one, a water content of 80 percent of the wet weight, and organic carbon as 40 percent of the dry weight, carbon is computed to be 0.08 times the volume. This common type of approximation leads to highly inaccurate biomass carbon estimates and points out the need for a rapid and accurate determination of biomass the result of which are values directly expressable in mg-C/m^2. For this reason direct carbon analysis of freeze-dried plankton was pursued. Measurement of carbon by high temperature dry combustion represents a rapid and accurate estimation of the zooplankton biomass.

II. METHODS

Carbon comprises close to 50% by weight of the organic matter in living organisms (Curl, 1962). Platt *et al.* (1969) found a high degree of correlation ($r = 0.94$) between the carbon content and calorific equivalent of marine zooplankton. Thus, organic carbon analysis may provide the most sensitive and reliable test of the energy content of the biogenous material in marine organisms. Its measurement should be equatable to the biomass of zooplankton. Despite the abundance of carbon, it is one of the most difficult elements to measure. For example, the presence of carbonate (considered inorganic carbon) in many marine organisms provides an added complication in measuring organic carbon. Curl (1962) points out that washing plankton with distilled water to remove chloride in interstitial seawater which can also interfere with carbon analyses can result in an appreciable loss for water-soluble carbon containing compounds from the organisms.

A. BACKGROUND OF CARBON ANALYSES

If seawater is filtered through a membrane filter on the order of 0.45 μm pore size, the organic carbon retained on the filter is referred to as "particulate carbon." The organic carbon passing through the filter is termed "dissolved". The particulate fraction is small and in oceanic waters rarely exceeds a few percent of the total organic carbon (Sharp, May, 1973) and at times the carbon content is determined on unfiltered samples. Thus, for evaluating carbon analysis of zooplankton it is useful to discuss literature on dissolved organic carbon and that of total organic carbon in seawater in the same context.

1. Dissolved Organic Carbon

Several methods for the determination of dissolved organic carbon (0.1 to 20 mg/l) have been used. Analysis of organic carbon in seawater is hindered by the existence of large quantities of inorganic salts which make the organic constituents minute in comparison. Accepted methods are generally based on wet oxidation of carbon by acid dichromate. Menzel and Vaccaro (1964) developed an analysis of dissolved carbon as an adaptation of a method by Wilson (1961) which consists of the wet oxidation of 1 - 5 ml of filtered seawater by potassium persulfate in a sealed glass ampoule after inorganic forms of carbon have been removed. The samples were subsequently flushed through a nondispersive infrared CO_2 analyzer. Approximately 100 samples can be analyzed in a single day with a precision of ±0.1 mg/l using a sample volume of 5 ml. Menzel (1967), and Williams and Gordon (1970) have also used this method for determination of dissolved carbon in the deep sea while Williams (1967) modified the procedure somewhat for sea surface chemistry. The Menzel and Vaccaro analysis was slightly modified and is now considered a standard analysis in seawater (Strickland and Parson, 1968). Morris and Foster (1971) utilized ultraviolet photoxidation of one liter samples followed by gravimetric estimation of the liberated carbon dioxide. The estimated precision by this method is ±5%.

2. Total Organic Carbon

Van Hall *et al* (1963) proposed a method for analysis of organic carbon in aqueous solutions by high temperature combustion. In their method, liquid samples were injected into a 950°C furnace and carbon was oxidized to CO_2 in an oxygen atmosphere. The resulting gas, after removal of water, was measured in an infrared analyzer. Their method

was not useable at carbon concentrations below 2 mg/l nor was it tested extensively with solutions of high salt concentrations. It has not been used for oceanographic work because of these limitations. Wangersky (1965) began development of an analyzer for use with seawater, similar to that of Van Hall *et al* (1963). Sharp (March, 1973) has developed a prototype for a high temperature combustion method for liquid samples. Oxygen is purified and used as a carrier gas. The combustion products pass through a condenser and $Mg(ClO_4)_2$ drying column into a Beckman IR-215 nondispersive infrared analyzer with CO_2-filled detectors and cells 34.3 cm in length.

Sharp and Wangersky (Sharp, March, 1973) have suggested that the most accurate method for measurement of organic carbon in seawater should involve high temperature combustion rather than wet chemical oxidation. The average precisions of the two methods are comparable and averages of errors are 5.0 - 5.5%; however the standard method of persulfate oxidation measures an average of 78% as much carbon as the high temperature method (Sharp, March, 1973). The works of P.J. Williams (1969) and P.M. Williams (1969) also seem to give evidence for incomplete analysis by the persulfate oxidation method.

Gordon and Sutcliffe (1973) have studied the feasibility of combusting freeze-dried seasalt at 730°C in a commercial elemental analyzer (Perkin-Elmer Model 240 Elemental Analyzer). This instrument was selected because it has a large sample volume capacity and combusts samples in pure oxygen. The combustion products (N_2, CO_2 and H_2O) are subsequently measured by a series of thermal conductivity detectors separated by appropriate scrubbers. Each complete analysis takes twelve minutes. The error of this carbon method was estimated to be

about 8%, somewhat greater than that reported by Sharp (March, 1973) for both his high temperature and wet oxidation methods.

Hewlett-Packard has developed a system for the quantitative analysis of particulate samples using a commercial carbon, hydrogen, and nitrogen analyzer and calculator system. This model (185B) provides a reproducible dry combustion method for the rapid oxidation of carbon in organic matter to gaseous products which are then determined by gas chromatography. The system was operated at sea and handles five samples per hour. Furnace temperatures reach 1100 ^{o}C and helium is used as the carrier gas (Atlantic Oceanographic Laboratory Report No. BI-R-73-14).

3. Particulate Carbon

a. In Sediments

"The most satisfactory method for analysis of the total carbon content of sediments of sedimentary rocks appears to be combustion of a sample at temperatures exceeding 1500 ^{o}C in an atmosphere of dry, CO_2-free oxygen. Such temperatures can be reached in high-frequency induction furnaces in which the sample is mixed with iron and heated". Similar to seawater analyses, depending on the amount of CO_2 evolved, combustion gases are then passed through a gasometric analyzer, through absorption trains for gravimetric analysis, or detected by nondispersive infrared CO_2 analyzers or thermal conductivity sensors (Carver, 1971). The LECO Carbon Analyzer, used for carbon analysis of plankton in this report, uses the high frequency induction furnace and a thermal conductivity sensor and has been used in sediment studies (Andrews, personal communication).

b. In Seawater

The method of Menzel and Vaccaro (1964) for determination of particulate carbon consists of concentration of the particulate matter from a one to four liter sample on a glass fiber or membrane filter, combustion in an automated furnace at 800 ^{o}C in the presence of CuO, using oxygen as a carrier, and the detection of the resulting CO_2 by infrared absorption. The precision of this method is 10 µg in a range of 0 - 500 µgC. Approximately six samples can be analyzed in an hour (Menzel and Vaccaro, 1964).

c. In Zooplankton

The total carbon content of the major taxonomic groups in net zooplankton from the upper 500 m of the Sargasso Sea off Bermuda was determined by Beers (1966). Samples of approximately 0.3-2.5 mg were combusted in a carbon analyzer furnace, the CO_2 liberated was collected, and the carbon determined by infrared analysis as in the Menzel and Vaccaro method.

Curl (1962) also analyzed total carbon in 19 species of marine organisms and mixed collections. Weighed, oven-dried samples or standards were placed in zirconium-ceramic crucibles together with one gram each of low-carbon iron chips and fine granular tin. Analyses consisted of combustion within the crucibles in the induction furnace (temperatures up to 2000 ^{o}C) and subsequent measurement of evolved CO_2 in a gas burette. The average sample was 20 mg of dry weight, and the carbon concentrations were from 6.6 to 46.8% of dry weight. According to Curl (1962), the advantages of rapidity and ease of operation of the induction combustion method for carbon are offset to some extent by decreased accuracy. However, the LECO instrument used in the author's

study which has an induction furnace and thermal conductivity sensor, was both rapid and accurate.

B. APPARATUS DESCRIPTION

1. Operation (after LECO instruction manual, 1974)

The instrument used for particulate carbon analyses in this study was the LECO (Laboratory Equipment Corporation) High Frequency Induction Furnace and the LECO 70 Second Carbon Determinator (Figure 1). In the method, a sample of known weight (0 - 100 mg) is placed in a ceramic crucible to which is added roughly 1 g of iron accelerator and 0.7 g of copper accelerator. The crucible containing the sample is then placed in the high frequency induction furnace within a combustion tube through which oxygen is passed. Since nearly all organic substances have high dielectric properties and are poor conductors of magnetic flux (Curl, 1962), samples must be heated indirectly through the use of the accelerators mentioned previously. The carbon in the sample is converted to CO_2 at temperatures in excess of 1600 ^{o}C. Metal oxides either remain in the crucible or are filtered out in a dust trap, while sulfur gases are absorbed in a trap containing manganese dioxide. Any carbon monoxide formed is converted to CO_2 in a heated catalyst tube. The dust trap, sulfur trap, catalyst tube and heater are mounted on the side of the induction furnace, as seen in Figure 1 . Moisture is removed in a Dehydrite trap which is mounted on the determinator. Both CO and moisture, is allowed to pass into the determinator, will cause erroneous results.

The carbon dioxide formed and the carrier oxygen are collected in a cylinder. The thermal conductivity of the gas mixture contained in the cylinder is measured by a thermistor type conductivity cell

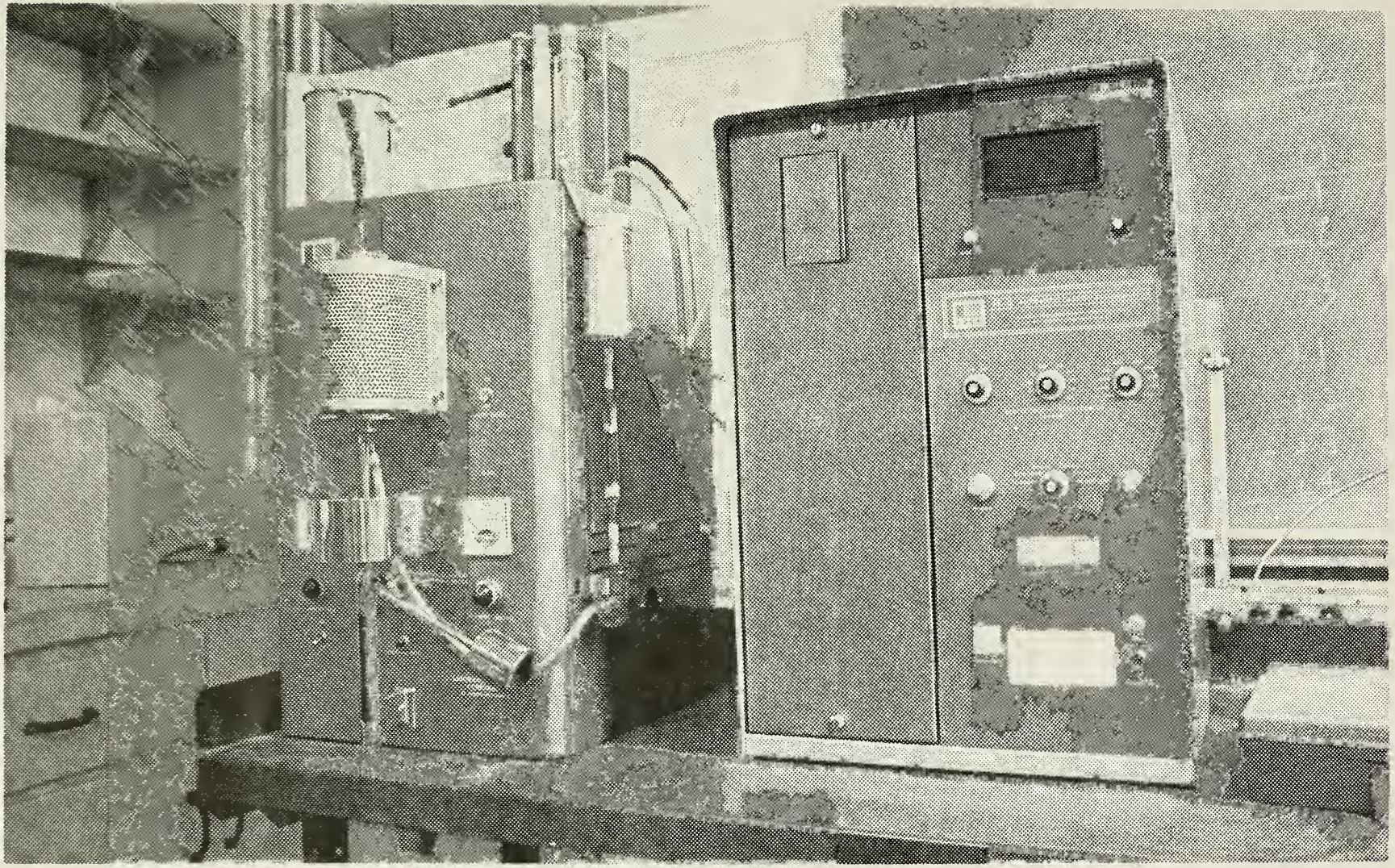


Figure 1. LECO High Frequency Induction Furnace (left) and LECO 70 second Carbon Determinator (right).

(See Appendix B). The output of the thermal conductivity cell is read on a special DC digital voltmeter. With the source oxygen in the cylinder, the thermal conductivity cell is balanced to yield 0.000 output as indicated on the digital voltmeter (DVM). The determinator utilizes the difference in thermal conductivity between oxygen and carbon dioxide. With the instrument thus balanced, the output of the thermal conductivity cell is indicated on the DVM and is proportional to the amount of CO_2 in the cylinder, assuming there are no other gases present in significant concentration which would affect the DVM reading.

The preamp, bridge circuit, and cylinder are housed in a temperature controlled oven where the temperature (45 ^{o}C) is set above ambient to eliminate variations which would cause an unbalance of the thermal conductivity cell or preamp. All the timing functions are controlled by solid state timers and reliable relays.

2. Maintenance

The only routine maintenance items performed after each run (20 - 30 samples)were the replacement of Dehydrite, glass wool, and metal screens in the moisture trap mounted on the side of the determinator and the cleaning of the dust trap and combustion tube found on the induction furnace. Another moisture trap for the oxygen supply is mounted on the side of the determinator. Its Ascarite, Dehydrite, and glass wool were replaced about every fifth run.

3. Accuracy and Precision

To achieve reliable and consistent results with the LECO Carbon Analyzer several conditions must be met. The instrument must be turned on three to four hours prior to the start of the analysis to allow metal and glass components to reach thermal equilibrium. An electronic

checkout and apparatus blanking process are required before analyzing any samples in each run to ensure that the instrument is calibrated correctly (See Appendix C). The samples to be analyzed must be dry since moisture will affect the thermal conductivity sensor. Finally, in the analysis of a sample the induction furnace plate current should go to between 400 and 500 milliamperes, which indicates that enough current has been attained to induce sufficient heating for good combustion.

Steel rings of 1 g each (less than 1% carbon) are recommended standards for the LECO Carbon Analyzer. During the course of the author's research, these steel rings were used to determine the accuracy and precision of this high-temperature dry combustion method for measuring carbon. On almost all runs in each type of experiment, one to several of the steel rings were analyzed for their carbon content. Since each steel ring represents a 1 g sample, the DVM readout is a direct expression of percent carbon. The estimates of accuracy and precision were found to be different for each type of steel ring analyzed. Three steel rings of different carbon content (0.074, 0.383, 0.854% C) were used. The compiled data for the tests and computations are given in Table I.

The lowest carbon steel ring (0.074% C) demonstrated the largest inaccuracy. The percent average error, a measure of accuracy, was computed to be ±10.4%, and the percent average deviation was found to be ±11.4% (Masterton and Slowinski, 1970). This seems, at first glance, to be very disappointing. However, a more realistic test for accuracy and precision on organic substances from 10 to 50 mg was the 0.383% C steel ring. Computed estimates of percent average error and deviation were ±3.1% and ±2.6% respectively. The 0.854% C steel ring yielded

TABLE I

ACCURACY AND PRECISION IN STEEL CALIBRATION RINGS

0.383 %C

DVM	Error	Deviation
.380	.003	.005
.383	.000	.008
.386	.003	.011
.396	.013	.021
.381	.002	.006
.363	.020	.012
.365	.018	.010
.350	.033	.025
.406	.023	.036
.372	.011	.003
.365	.018	.010
.374	.009	.001
.370	.013	.005
.378	.005	.003
.363	.020	.012
.373	.010	.002
.368	.015	.007
.386	.003	.011
.378	.005	.003
.376	.007	.001
.368	.015	.007

0.383 %C
(con.)

	DVM	Error	Deviation
	.368	.015	.007
	.362	.021	.013
	.387	.004	.012
Sum	8.630	.271	.224
Mean	.3752	.0118	.0097

0.854 %C

	.861	.007	.001
	.856	.002	.006
	.855	.001	.007
	.871	.017	.009
	.856	.002	.006
	.853	.001	.009
	.840	.014	.022
	.877	.023	.015
	.871	.017	.009
	.841	.013	.021
	.857	.003	.005
Sum	9.8411	.100	.110
Mean	.862	.0091	.01

0.074 %C

	DVM	Error	Deviation
	.073	.001	.003
	.074	.000	.004
	.069	.005	.001
	.077	.003	.003
	.054	.020	.016
	.056	.018	.014
	.080	.006	.010
	.076	.002	.006
	.059	.015	.011
	.081	.007	.011
Sum	.699	.077	.079
Mean	.0699	.0077	.0079

Error = observed - true value

Deviation = observed - average value

.383% C Steel Ring

$$\text{\% Average deviation (from mean)} = \frac{.0097393}{.3752174} \times 100 = 2.595\%$$

$$\text{Precision} \simeq \pm 2.6\%$$

$$\text{\% Average error} = \frac{.011783}{.383} \times 100 = 3.0765\%$$

$$\text{Accuracy} \simeq \pm 3.1\%$$

.854% C Steel Ring

$$\text{\% Average deviation} = \frac{.01}{.862} \times 100 = 1.16\%$$

$$\text{Precision} \simeq \pm 1.2\%$$

$$\text{\% Average error} = \frac{.0090909091}{.854} \times 100 = 1.064\%$$

$$\text{Accuracy} \simeq \pm 1.1\%$$

.074% C Steel Ring

$$\text{\% Average deviation} = \frac{.0079}{.0699} \times 100 = 11.302$$

$$\text{Precision} \simeq \pm 11.3\%$$

$$\text{\% Average error} = \frac{.0077}{.074} \times 100 = 10.405\%$$

$$\text{Accuracy} \simeq \pm 10.4\%$$

even better results of ±1.1% average error and ±1.2% average deviation. For the purpose of estimation of zooplankton biomass by the use of the carbon analyzer, the author considers the reported estimates of accuracy and precision for the mid-range (0.383% C) steel ring to be the most applicable. That is, the average percent error (accuracy) and average deviation (precision) were both determined to be approximately ±3%.

4. Designed Uses and Applications

It must be remembered that the LECO Carbon Analyzer was designed for sensitive, low carbon measurements (<10%) as evidenced by the recommended use of low carbon steel rings as standards. This carbon analyzer has been adapted to measuring high (≈50%) carbon in organic substances. The use of small samples, on the order of 10 mg of dry zooplankton for the estimation of biomass, allowed the resultant measurement of carbon in these samples since the DVM readouts were in the same order of magnitude as the steel rings.

C. ATP - CARBON ANALYSIS

ATP was measured by the Holm-Hansen and Booth (1966) method as modified by Dr. Traganza for zooplankton analyses using a JRB Model 1 ATP Photometer. ATP was converted to ATP-C by the equation: 0.2382 ATP = ATP-C (Traganza, personal communication).

D. FREEZE-DRYING OF FIELD SAMPLES

Net plankton and laboratory samples (described in section III) were filtered onto Whatman GF/C glass fiber filters (0.45 μm pore size mesh) which had been precombusted at 450 - 500 ^{o}C for 2 - 3 hours. These samples were held in a freezer at -3 ^{o}C and later "freeze-dried" in the laboratory at -196 ^{o}C with liquid nitrogen. The glassware unit

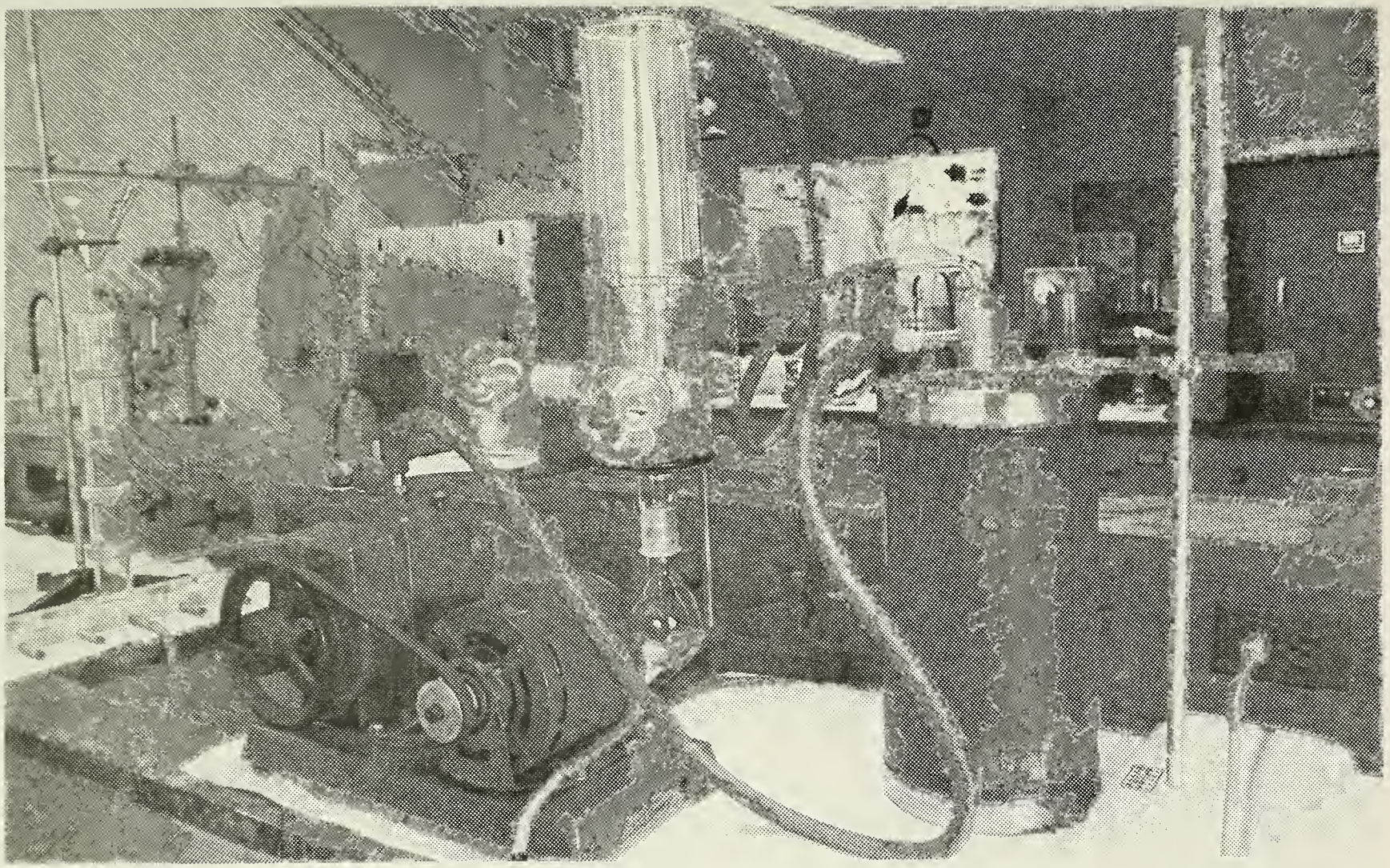


Figure 2. Freeze drying unit with liquid nitrogen cold finger trap, vacuum pump, and sample flasks which connect to the unit.

shown in Figure 2 employed the use of a vacuum pump (29.2" Hg); a liquid nitrogen cooled protective trap; a liquid nitrogen cold finger trap; a condenser (merely the outside surface of the cold finger); and a set of sample flasks which connect to the unit. A set of 10 to 15 glass fiber filters and samples were freeze-dried for approximately 20 hours to ensure complete dryness of the sample before carbon analysis. Replenishment of the liquid nitrogen was required approximately every three hours. The resultant total use for a twenty hour freeze-drying process was up to 40 liters of nitrogen at a approximate cost of twelve dollars. Unfortunately, this procedure is quite costly and time consuming. It appears to be the single most important limiting factor in the presented carbon analysis scheme.

E. REGRESSION ANALYSIS

A tape program was used for all fitting of curves to experimental data by the method of "Best Fit" (Wang Laboratories, Program S.107-7.3) which, according to the program description, calculates the equation of the line by minimizing the squares of the perpendicular deviations of the points from the line. This line takes into account deviations due to the variability in both X and Y value, in contrast to the method of "Least Squares," which minimizes the squared deviations from the line in the Y-direction or X-direction. This is especially helpful with the experimental data in section III since the X (*i.e.* an approximated mass) and Y (*i.e.* DVM readout) values are not considered absolute and are subject to deviations. Figures 18-21 show the output of this program and the "best fit" line which fits the presented data. Neither X nor Y can equal zero when entering data points to be plotted. Very small values of X and Y were used with apparatus blanks made both prior to and following each combustion series.

In addition to plotting the axes, data points, and line of "best fit" this program types out the number of data points plotted (n) and the correlation coefficient (r) of the line of best fit. This coefficient is based on the following formulas:

$$Y - \overline{Y} = -\frac{1}{2\Sigma xy}[\Sigma x^2 - \Sigma y^2 - \sqrt{(\Sigma x^2 - \Sigma y^2)^2 + 4(\Sigma xy)^2}](X - \overline{X})$$

where:

$$\Sigma x^2 = \Sigma X^2 - \frac{(\Sigma X)^2}{n} \quad ; \quad \Sigma y^2 = \Sigma Y^2 - \frac{(\Sigma Y)^2}{n}$$

$$\Sigma xy = \Sigma XY - \frac{(\Sigma X \Sigma Y)}{n}$$

$$r = \frac{\Sigma xy}{\sqrt{\Sigma x^2}\ \sqrt{\Sigma y^2}}$$

The equation used to calculate the line of best fit was $Y = a + bX$ where a and b are the following relationships:

$$b = -\frac{1}{2\Sigma xy}[\Sigma x^2 - \Sigma y^2 - \sqrt{(\Sigma x^2 - \Sigma y^2)^2 + 4(\Sigma xy)^2}]$$

$$a = \frac{\Sigma Y}{n} - b\frac{\Sigma X}{n}$$

III. EXPERIMENT DESCRIPTIONS

According to Chester and Riley (1971), the quantitative estimation of zooplankton biomass (and production) is a difficult task. "The estimation of biomass is perhaps the field calling most for standardization but at the present time no one method seems to offer the potential for widespread adoption" (Tranter, D.J. and Fraser, J.H., eds, 1968). In order to evaluate the measurement of zooplankton biomass using the LECO Carbon Analyzer, the following experiments were undertaken.

A. STANDARDIZATION

Since the LECO Carbon Analyzer was specifically designed for low carbon measurement in steel (*e.g.* less than 10% carbon in steel rings of one gram each), there was a question to its applicability to the measurement of carbon in relatively high carbon content compounds. An average of 50% carbon in dry weight is commonly assumed for pelagic marine invertebrates (Curl, 1962). Two organic compounds with nearly this carbon content were used in small amounts (0 to 75 mg) to stay within the detection limits of the analyzer.

1. Benzoic Acid

Benzoic acid is a fairly common chemical standard of known carbon content (68.8487%), as computed from the total molecular weight (Hodgman, 1957). Sharp (March, 1973) used benzoic acid solutions for standardization of his combustion analyzer. Combustion of low mass samples occurs nicely with copper and iron accelerators. Greater than 50 mg samples of benzoic acid, however, did undergo rapid combustion and small deflagrations did occur.

A series of three runs of various masses of benzoic acid was performed and the readouts of the digital voltmeter (DVM) were recorded. Each of the three runs occurred on a different day. The weight of each sample was determined on a Mettler microbalance (0.1 mg). The normal apparatus blanking process was accomplished prior to each run (See Appendix C).

2. Casein

Casein is a common protein with a carbon content of 53.13% (Heilbron, 1946), roughly paralleling the carbon content of marine invertebrates. The use of casein as a second standard was employed for two reasons. First, it was used to determine a second standard curve to compare with that of benzoic acid. Secondly, casein allowed the evaluation of possible contamination of the thermal conductivity detector by oxides of nitrogen.

The same procedure used with benzoic acid was applied to casein. A series of three runs of various masses of casein was performed, and the DVM values and weights were recorded. Each of the runs again was done on a different day. Combustion was complete on all but a few samples, as indicated by the furnace plate current meter. These samples were discarded.

3. Infrared Analysis

To further test the possible interference of gaseous products, specifically nitrogen oxides, an analysis of the combustion products collected in the cylinder of the carbon determinator was performed. A modification was made in the rear of the determinator to allow passage of the combustion products from the cylinder to a small 10 cm NaCl infrared cell (Figure 3). Analysis of each sample containing these

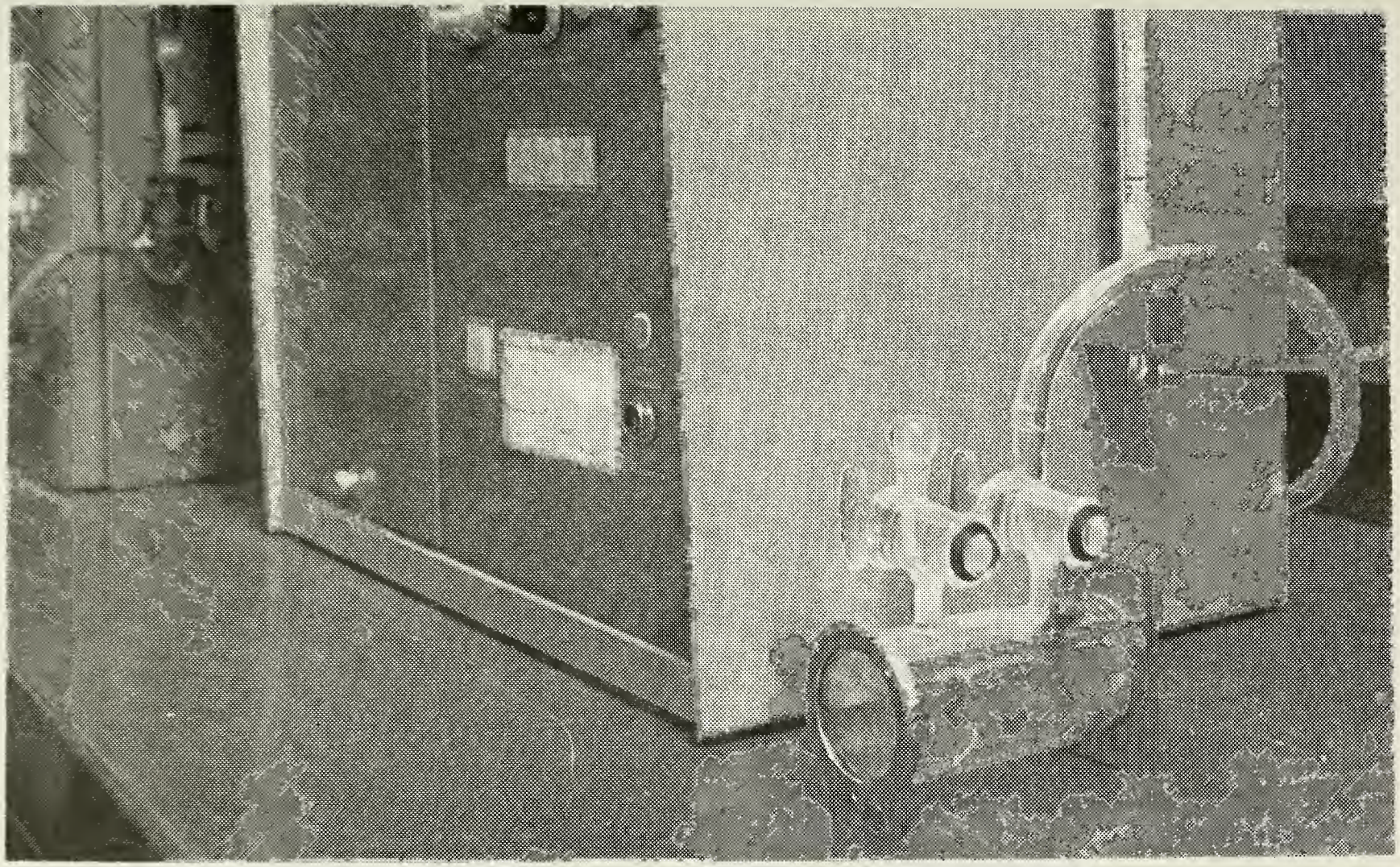


Figure 3. Infrared 10 cm NaCl cell adapted to LECO 70 second Carbon Determinator.

combustion products was performed on a Perkin-Elmer 337 Grating Infrared Spectrophotometer. Samples from one of the casein standardization runs and from field sample analyses were taken as specimens to be analyzed. The carbon evaluations for these samples are in Table III.

B. CARBON IN *Tigriopus californicus*

In order to adequately determine the applicability of the LECO Carbon Analyzer to zooplankton studies, a preliminary test to evaluate the carbon content of a single copepod species was made. "*Tigriopus californicus* is a harpacticoid, supra-littoral benthic copepod that is related to the pelagic, planktonic calanoid copepods. Convenience in choosing a test organism was considered, but at the same time it was important to use one that was similar to typical zooplanktonic species" (Baugh, 1974). The Copepoda comprise over 60% of the pelagic animal families and are, as such, the most common of the zooplankton in number. Populations of *T. californicus* were easy to obtain since they live in splash pools and occur exclusive of any other species along the west coast of California (Egloff, 1966). Field collections of natural populations of *T. californicus* were taken from splash pools above the mean high-water mark along the rocks that line the beach around Lover's Point at the southern end of Monterey Bay, California. Identification of the species was simplified due to the distinctive reddish-orange color and essentially homospecific nature of the catches. The animals were scooped from the pools with a number 10 plankton bucket (160 m mesh size).

Once collected, the copepods were kept in a plastic container at room temperature near a source of sunlight. The only source of nourishment for the copepods was the natural food in the seawater from the

splash pools. After allowing the collection of *T. californicus* to stand for approximately one week, the animals were fractionated into size groups with the use of a sieve column (Figure 4.) and small "Nitex" nylon screens of various size meshes. The sieve column was back filled with pre-filtered seawater prior to fractionation to maximize the effectiveness of the process.

This experiment was run twice. In the first experiment, three mesh sizes were employed: 297 μm, 177 μm, and 125 μm. The largest mesh size was placed at the top of the sieve column and so on, down to the smallest mesh size at the bottom. In the second experiment, as a result of lessons learned in size fractionation of this species, larger mesh size screens were used: 420 μm, 320 μm, 297 μm, and 250 μm. It was hoped that this approach might give answers as to the variation of carbon content in the different life stages of *T. californicus* as delineated by the different size fractions (see Figures 5(a) and (b).

After fractionation, the nylon screens, with hundreds of copepods of each appropriate size range, were placed in the freeze-drying unit (Figure 2.) for approximately 20 hours (minimum drying time may be less). Carbon analysis was done on the freeze-dried organisms which were carefully scraped into the ceramic crucibles. The crucibles were preweighed in order to determine the mass of each sample being analyzed. The mass and DVM reading of each sample were recorded in both experiments. The data from these experiments is given in Table IV.

C. ATP-CARBON TO TOTAL CARBON RATIO IN *Tigriopus californicus*

Two joint experiments were conducted with Dr. Traganza to examine the ATP-C to total C (carbon) ratio in the test organisn and to determine a method of finding the carbon present in living cells of organisms.

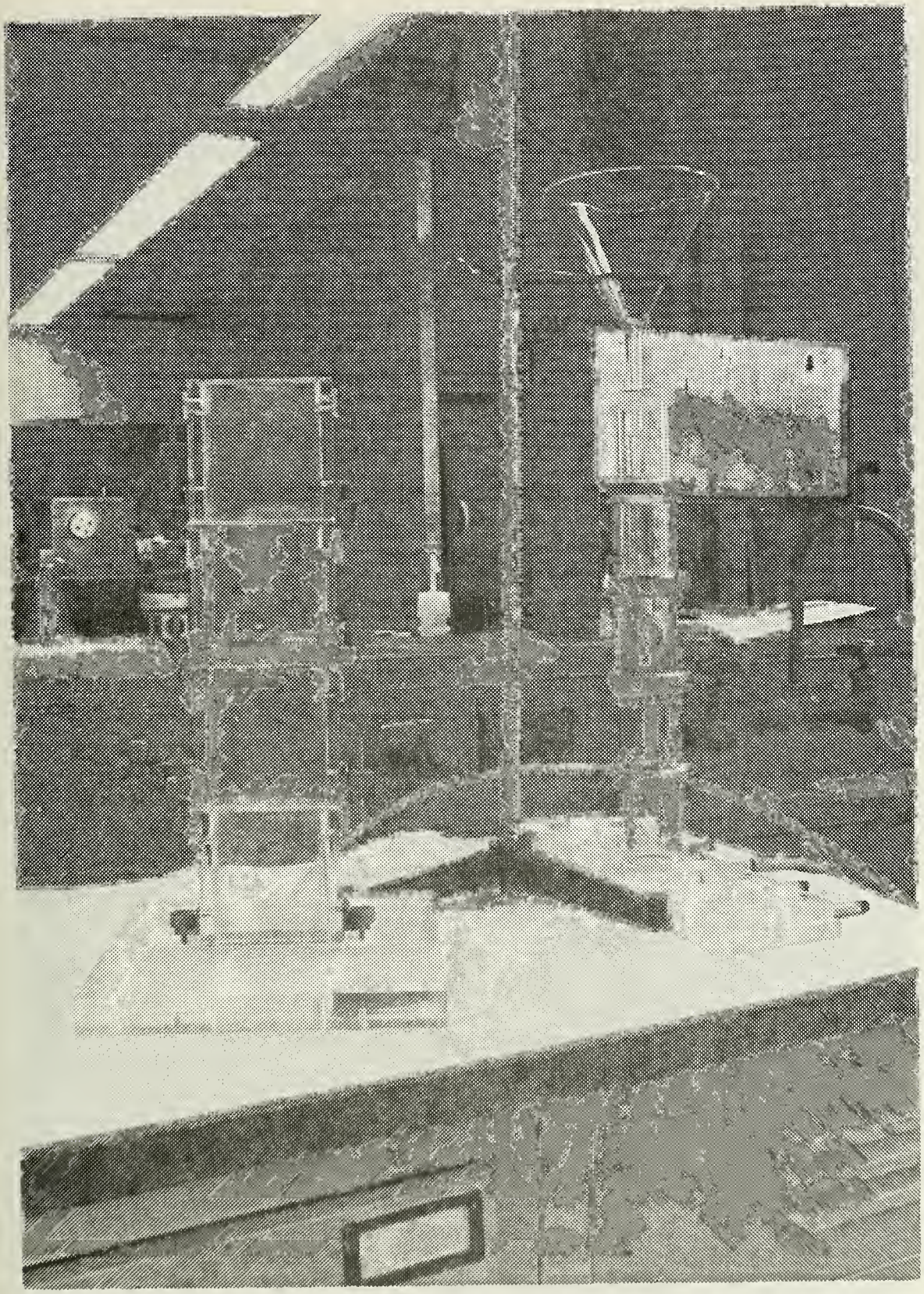


Figure 4. Sieve columns used for screening size fractions.

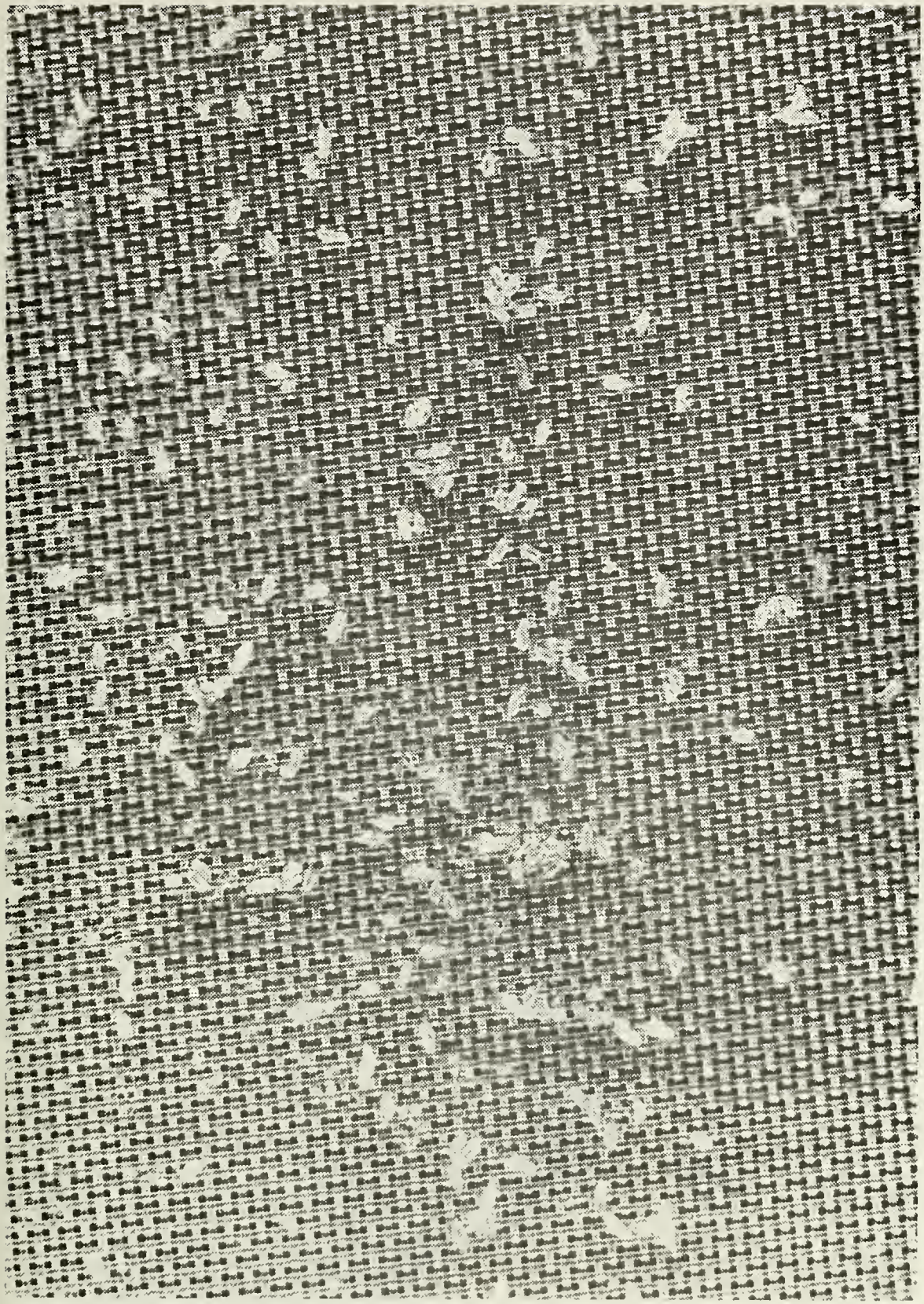


Figure 5(a). *Tigriopus californicus* sieved onto a 297 μm nylon screen.

Figure 5(b). Three size fractions of *Tigriopus californicus* after screening and placed into samples dishes.

Collections from natural populations of *T. californicus* were made in the same manner as mentioned in the previous experiment. The organisms were fractionated into size groups with the sieve column and Nitex nylon screens of various meshes. In the first experiment, two screen sizes were used: 420 μm and 297 μm. To obtain a better representation similar to the zooplankton net size fractions used in the field studies, a third screen of 250 μm mesh size was added for the second experiment. After fractionation, the organisms were washed from the screens into glass tissue homogenizers for ATP extraction in 40 ml hot (≈100°C) TRIZMA buffer solution (pH = 7.7). The "particulate" homogenate was captured on a 0.45 μm mesh Whatman GF/C (type C) glass fiber filter, at full vacuum (29.2" Hg). The filters were preburned for two to three hours at 450 - 500°C to remove organics. The oven and filtration unit are shown in Figure 6. ATP analysis was performed on 0.5 ml of a 2 ml aliquot (liquid) which was taken before and after filtration. The suspended particulate C in the before filtration aliquot was accounted for in the calculation of total C. Any non-ATP carbon which dissolved in the TRIZMA extract was not accounted for but was presumed to be insignificant. The particulate matter collected on the filter was freeze-dried and analyzed for carbon.

The first experiment consisted of one fractionation yielding six samples: three in the range of 297 to 420 μm; two greater than 420 μm; and one control filter containing no organisms. In this scheme the effect of filtration on ATP analysis was tested. Hence, two values of ATP-C were obtained for every one carbon determination. In the second experiment, however, the aliquot for ATP analysis was taken after fractionation and no correction was necessary for particulate C.

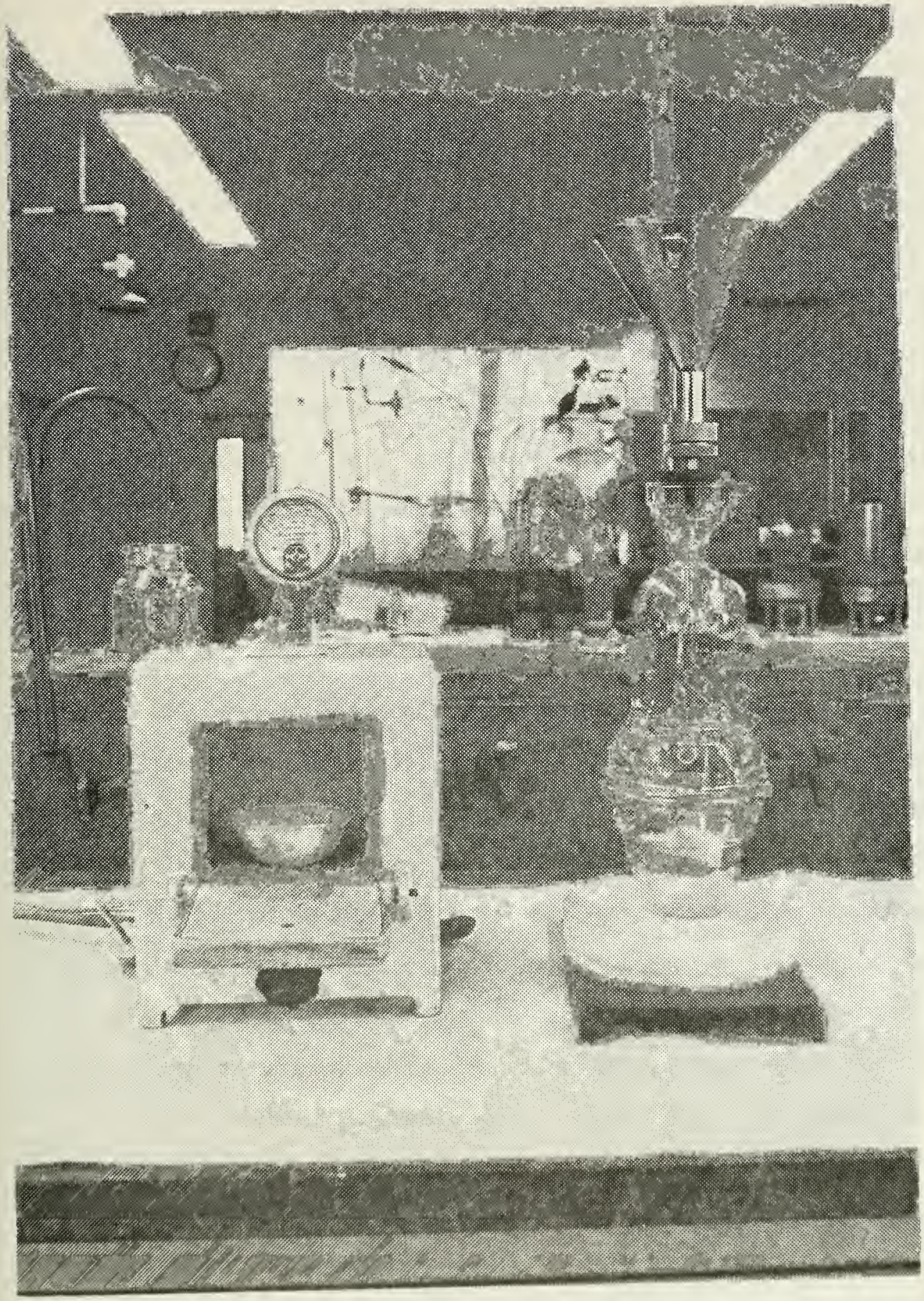


Figure 6. Oven for precombusting glass filters (left) and filtration unit used in ATP-C to total C experiment.

Therefore, two similar experiments on two different size fractions were performed. The data from these experiments is given in Table V.

D. ATP-C AND CARBON ANALYSES IN ASSOCIATION WITH FIELD STUDIES

The development of meaningful acoustic models related to oceanic food chains requires a knowledge of the variation of the biomass of planktonic organisms in the ocean. A series of five cruises in 1974 were made to evaluate experimentally the relationship of ATP-C to total carbon in zooplankton. ATP and carbon analyses were made on "net zooplankton" samples to determine living and total carbon in each catch as a measure of seasonal variability of zooplankton biomass. The first two cruises provided experience in the proper use of shipboard equipment and sampling apparatus. The following three cruises (May, July, and August) provided the basic data for the studies.

All cruises were conducted on board the R/V ACANIA (Figures 7(a) and (b)), the research vessel of the Naval Postgraduate School. The cruise area used in all studies is shown in Figure 8. Its center was approximately fifteen miles from the Monterey Coast Guard Pier and covered an area of about 80 square miles. This "deep-ocean" site was chosen to minimize confusion from neritic species. Once on station, a parachute drogue was set for a depth of 30 meters and placed in the water to serve as a water mass reference. All stations were made with reference to the drogue, as shown diagramatically in Figure 10(b). The use of the drogue was an attempt to allow sampling in a water mass with which the plankton moved. This eliminated horizontal advection effects so as to detect the intrusion of vertically migrating species at night. It also permitted an attempt to use a search pattern for biomass maxima which may be associated with "zooplankton patches". Substantial verification of this

Figure 7(a). R/V ACANIA at the Monterey Coast Guard Pier.

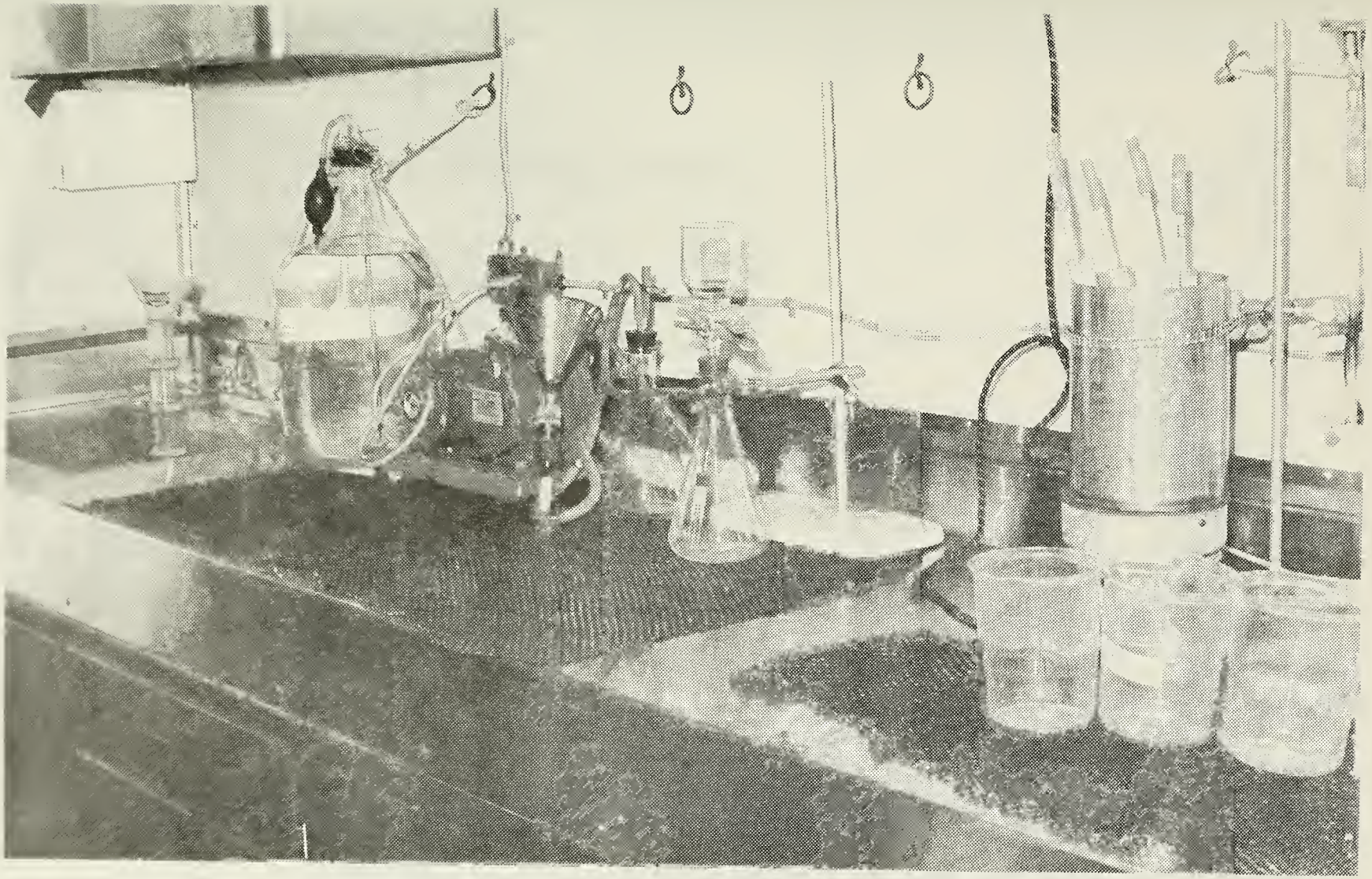


Figure 7(b). Laboratory aboard the R/V ACANIA. Carbon filtration apparatus on the left and ATP extraction equipment on the right.

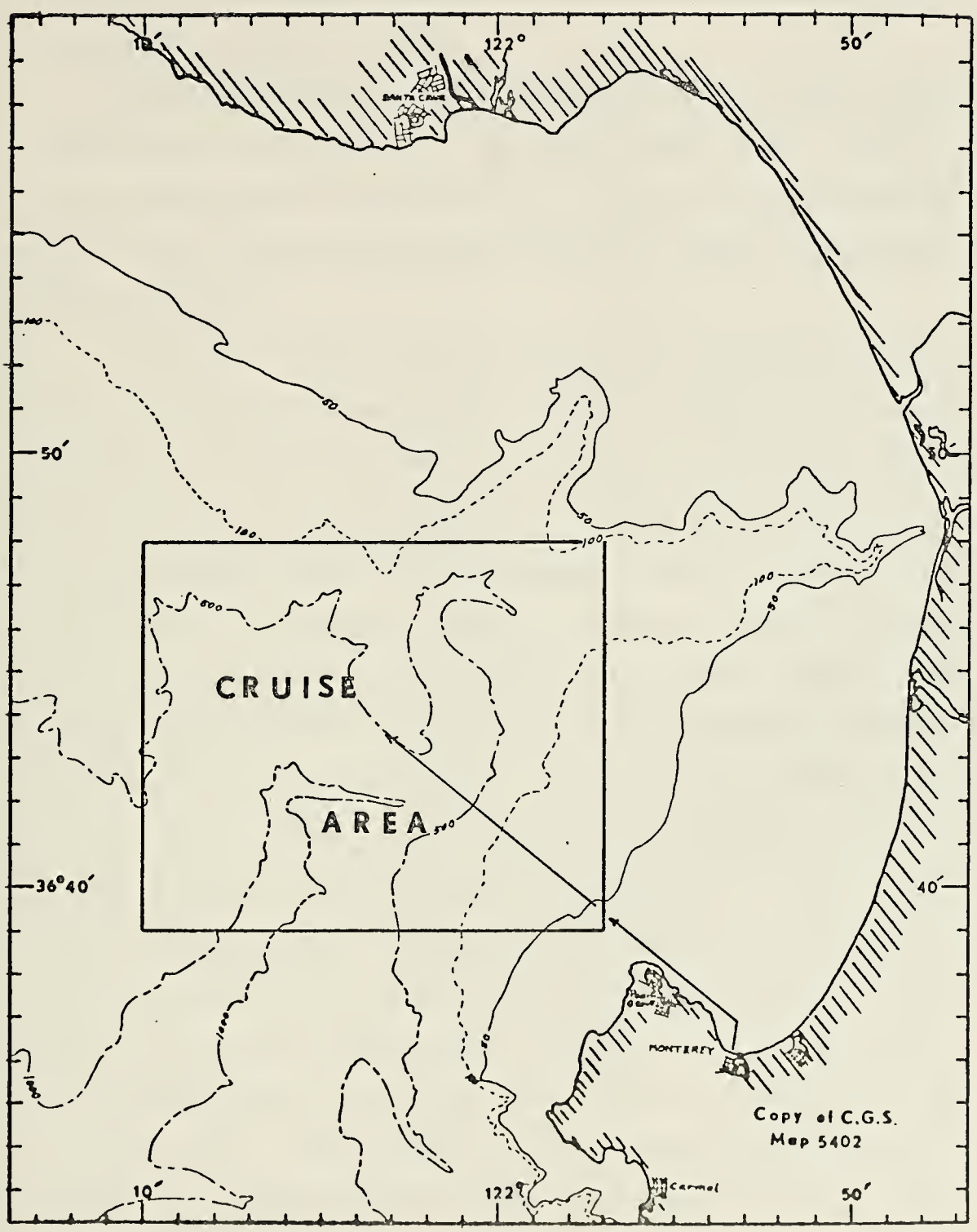


Figure 8. Cruise area for all stations

approach was obtained by T-S diagrams. The geographic plots of the drogue track and sampling positions for the cruises are displayed in Figures 9(a), 10(b), and 11(b).

The search pattern represented in the second cruise (July), shows an X-type station plot relative to the drogue (Figure 10(b)). In this case, the drogue migrated almost due north. The station positions demonstrated an almost ideal search pattern based on an optimum search technique under evaluation.

The sampling scheme consisted of an in-line multiple net vertical tow from 200 meters to the surface. The in-line system of nets contained five different mesh sizes with the coarsest net on top down to the finest at the bottom. The first generation net system (used on the first cruise in May) is one of several sampling schemes under evaluation by Traganza (see Figures 12(a) and (b)). The net system was attached to the hydrowire and run tail first to 200 meters and then back at 40 m/min. Each bucket was removed from each net. The five corresponding mesh sizes for nets #3, #6, #8, #10, and #14 were > 333 μm; 333-243 μm; 243-202 μm; 202-160 μm; and 160-102 μm. The net sample from the bucket was then concentrated on Nitex nylon screens with the sieve column. In some cases, the sample was split with a "plankton splitter" before being poured into the sieve column in order to lessen the bulk of organic material. The normal extraction and grinding routine followed for ATP analysis, and the particulate material collected on glass filters was frozen in the ship's freezer ($<-3^{o}C$). These samples were later freeze-dried and analyzed for carbon. ATP samples were also frozen until analysis ashore. In addition to the accumulation of total (*i.e.*, the sum of living plus dead) and living carbon data, other relevant oceanographic observations were

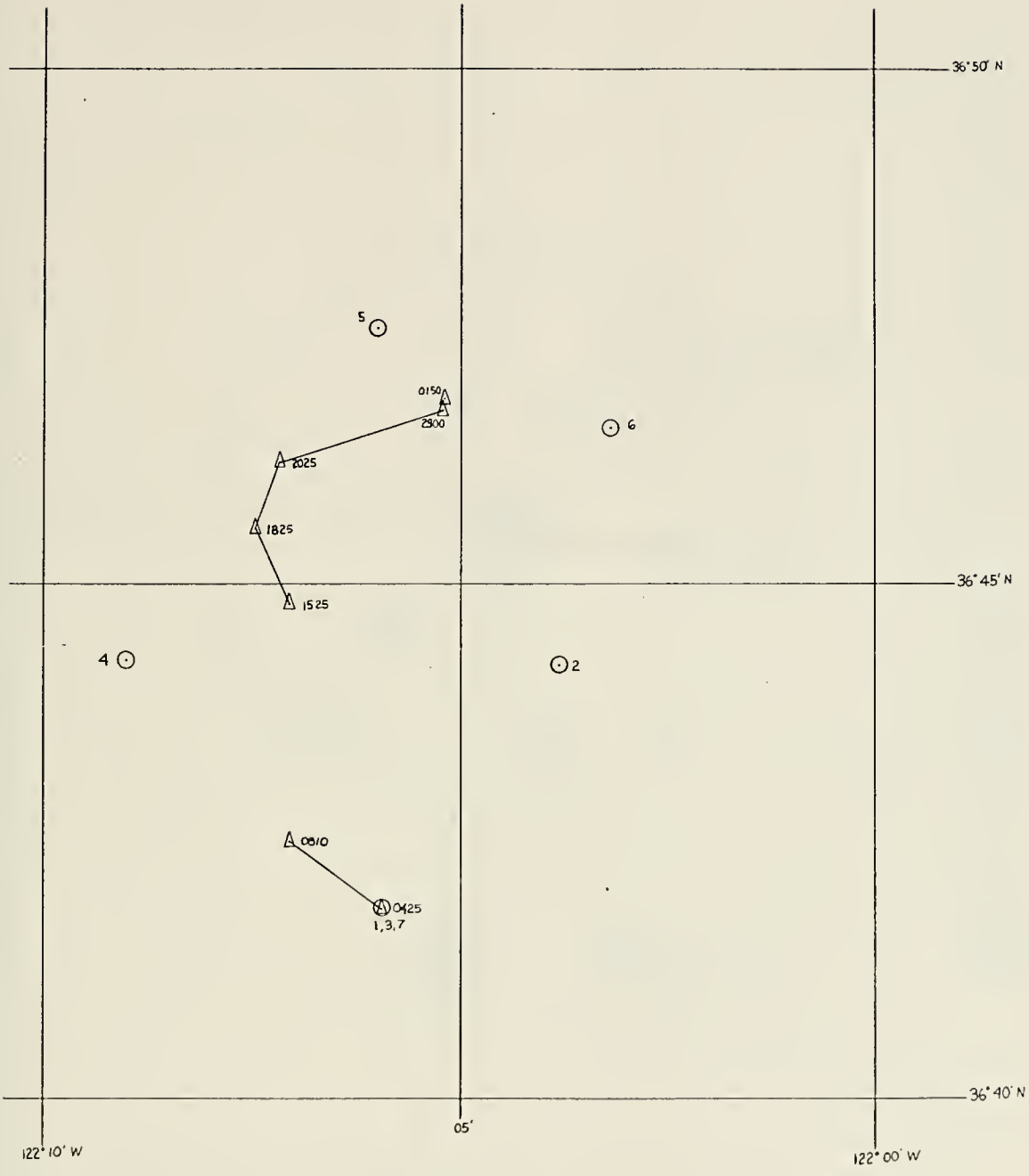


Figure 9(a). Drogue track and geographic station plot for May cruise (7403).

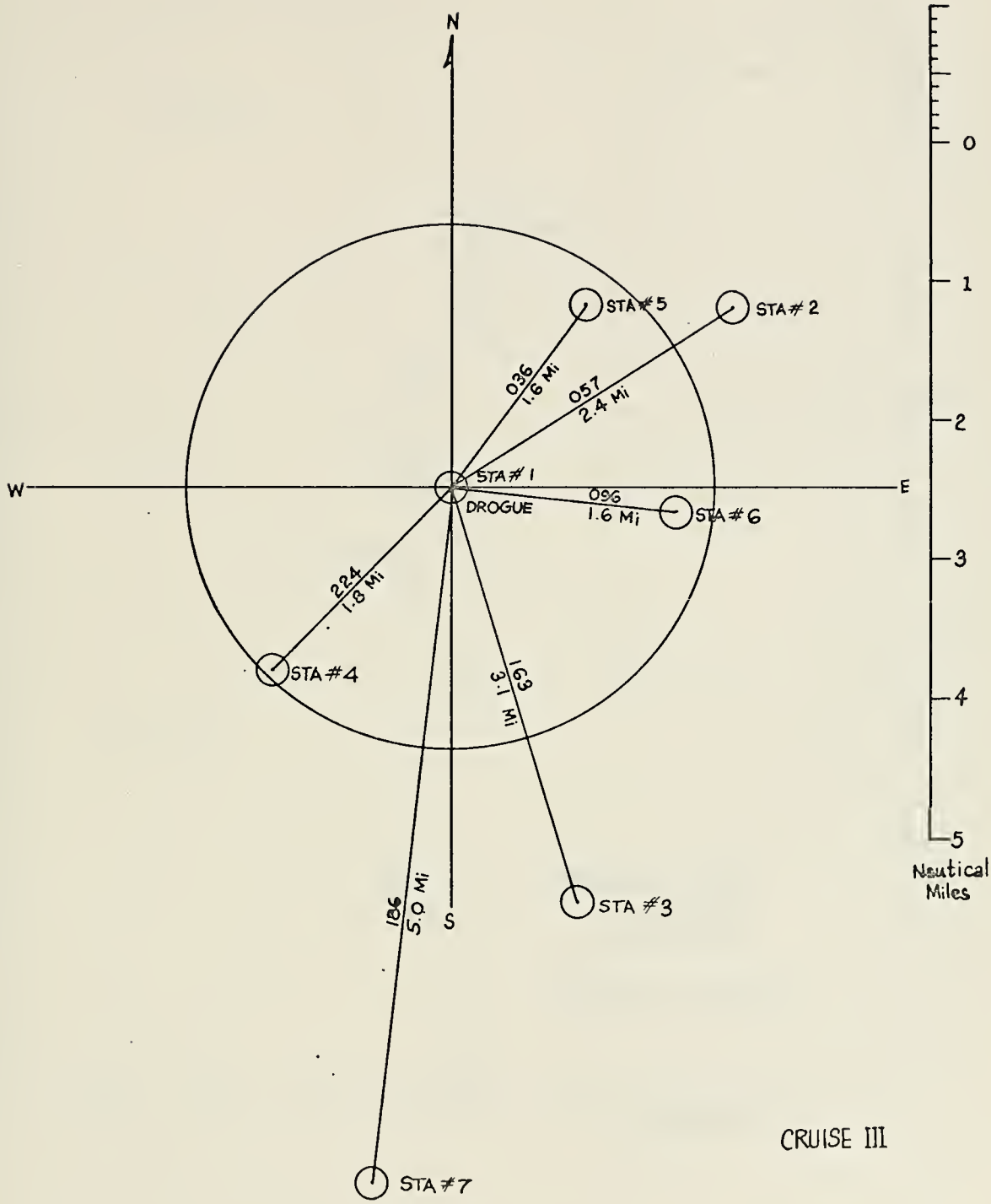


Figure 9(b). Water mass station plot relative to a drogue for May cruise (7403).

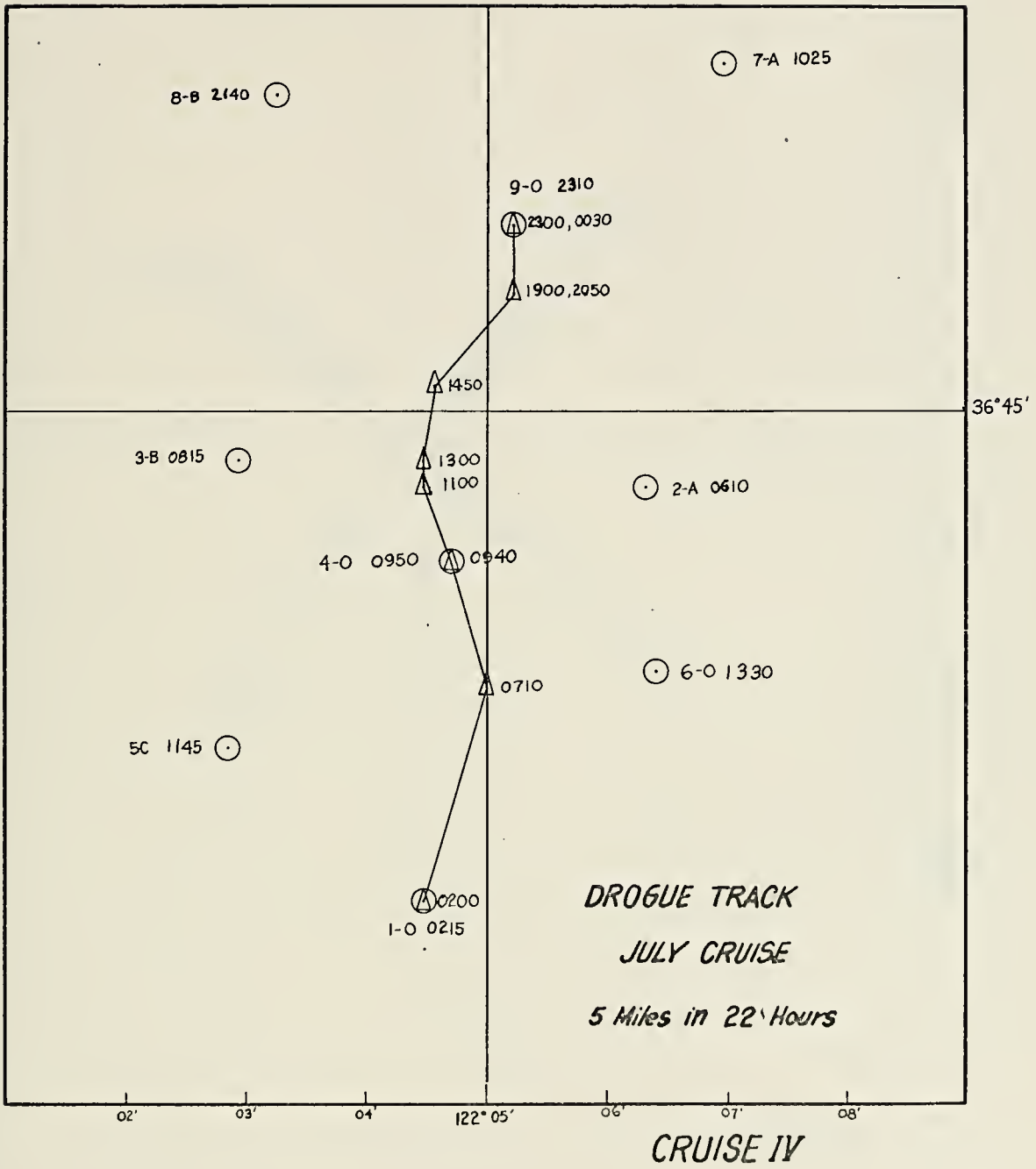
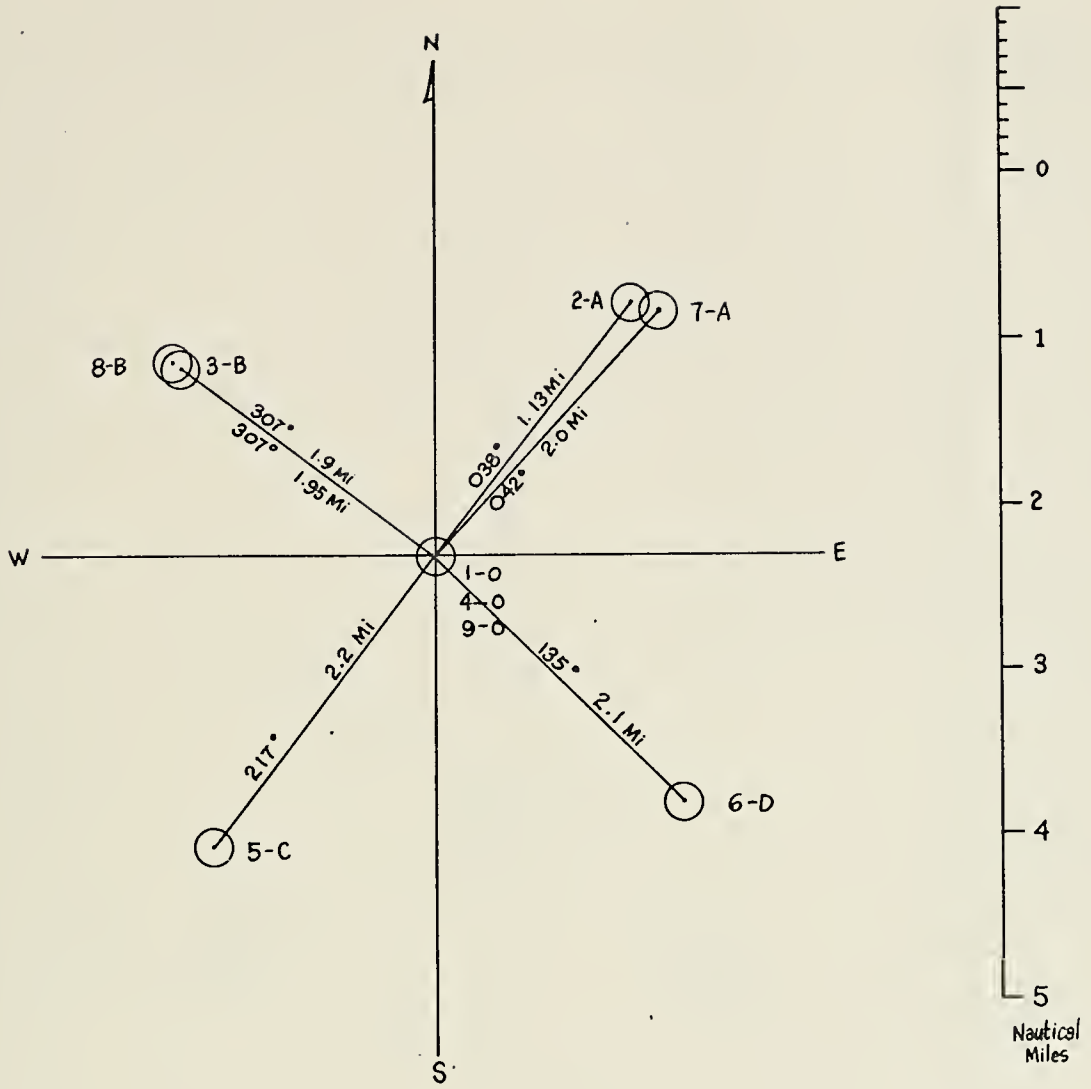


Figure 10(a). Drogue track and geographic station plot for July cruise (7404).



CRUISE IV

Figure 10(b). Watermass station plot relative to a drogue for July cruise (7404).

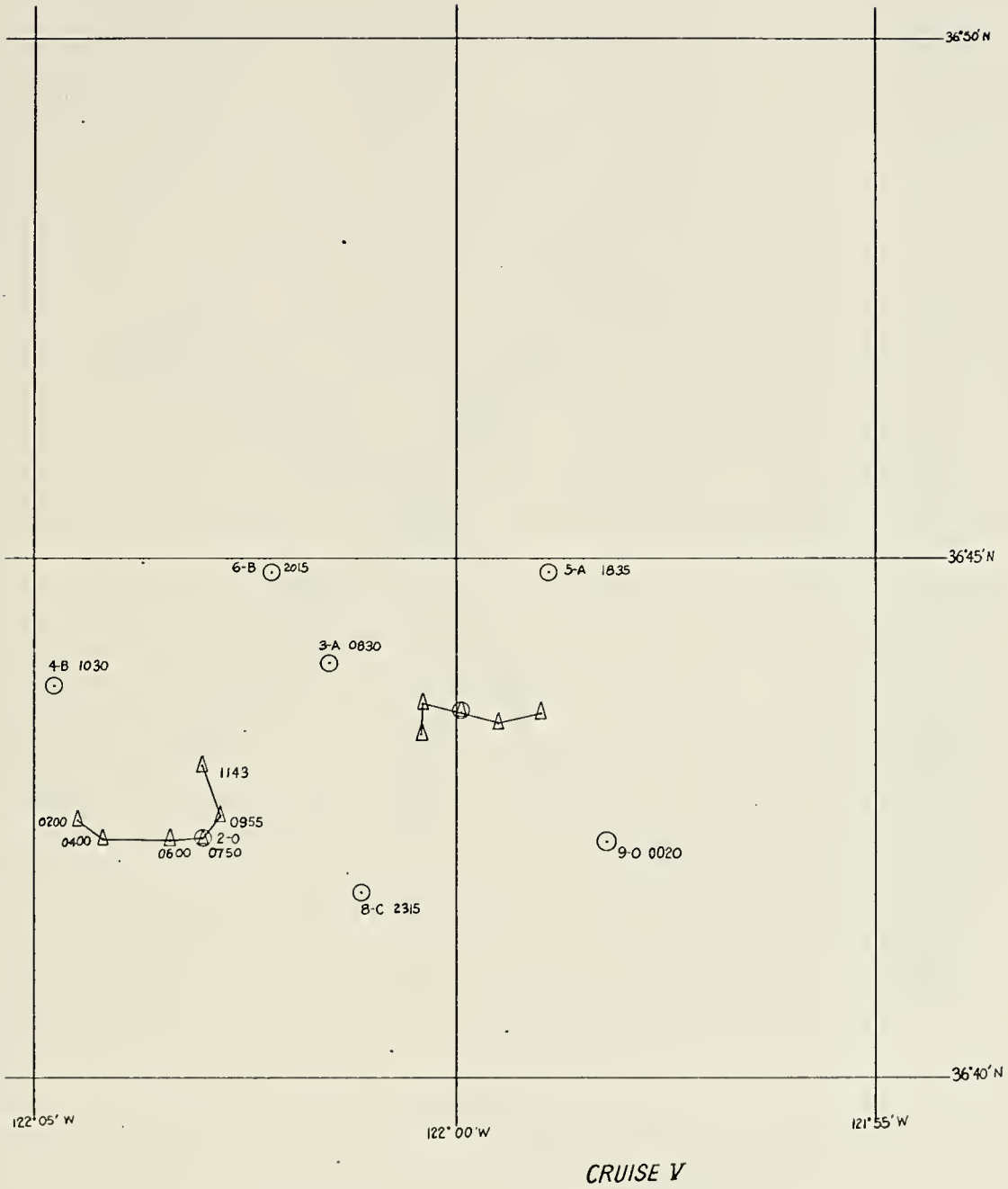
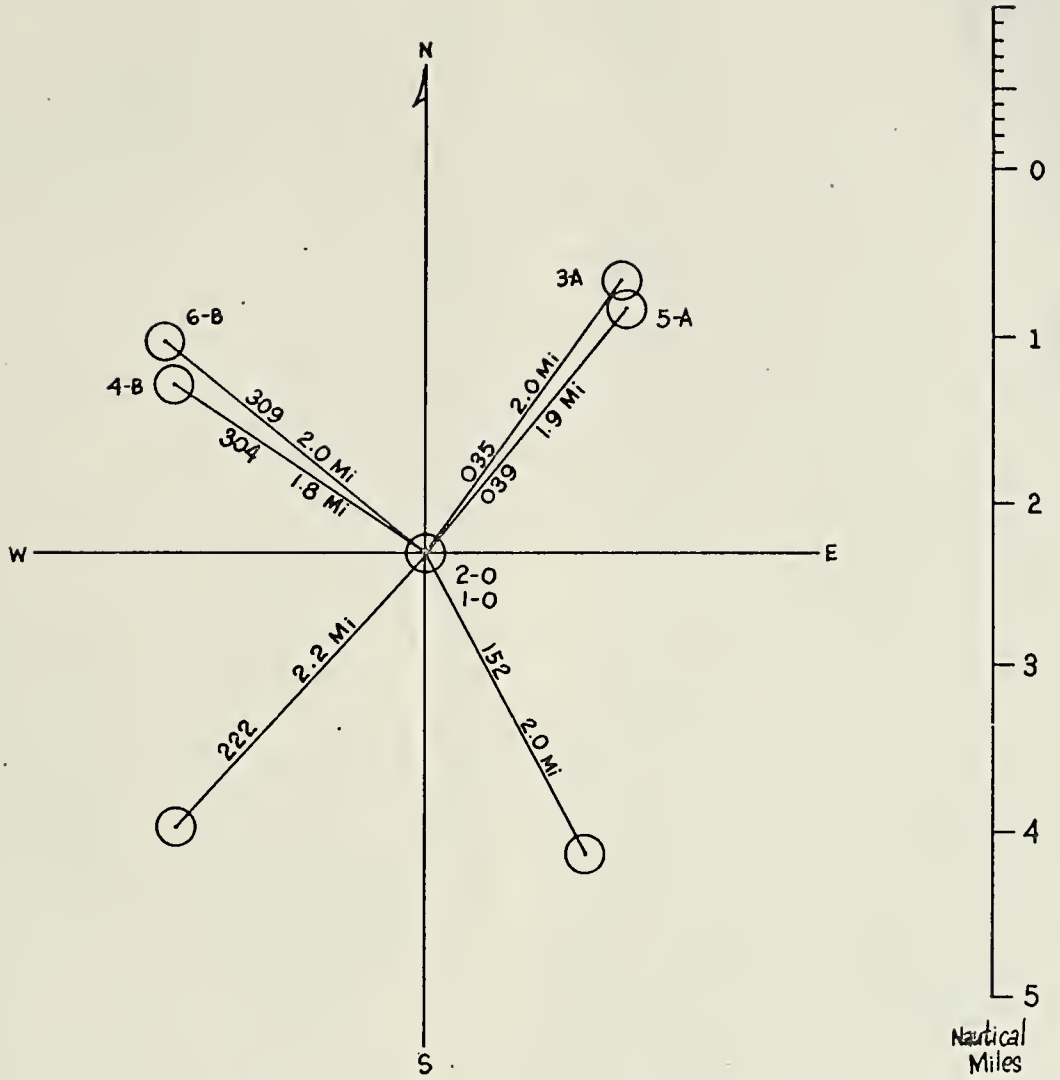


Figure 11(a). Drogue track and geographic station plot for August cruise (7405).



CRUISE V

Figure 11(b). Watermass station plot relative to a drogue for August cruise (7405).

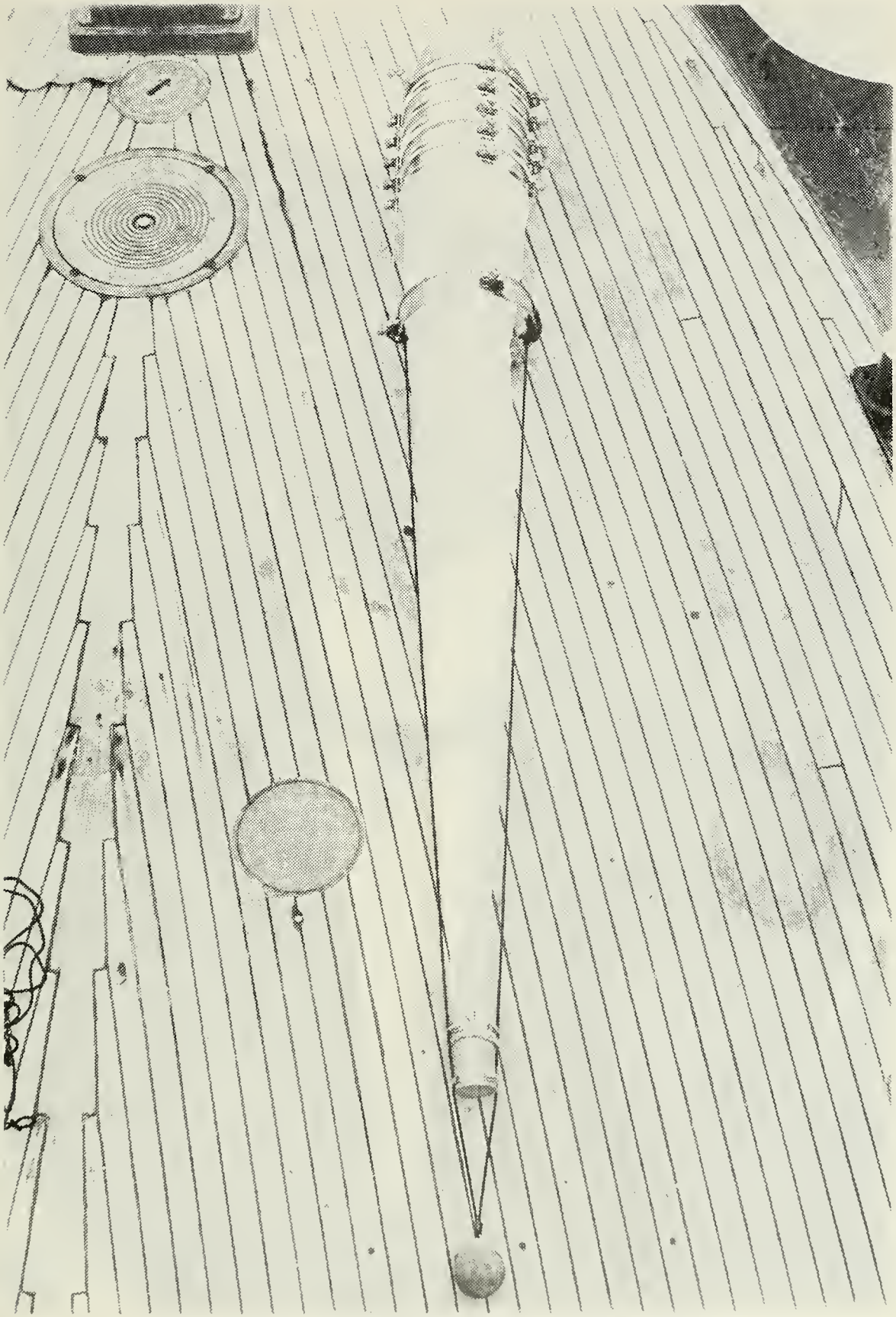


Figure 12(a). First generation net system on deck.

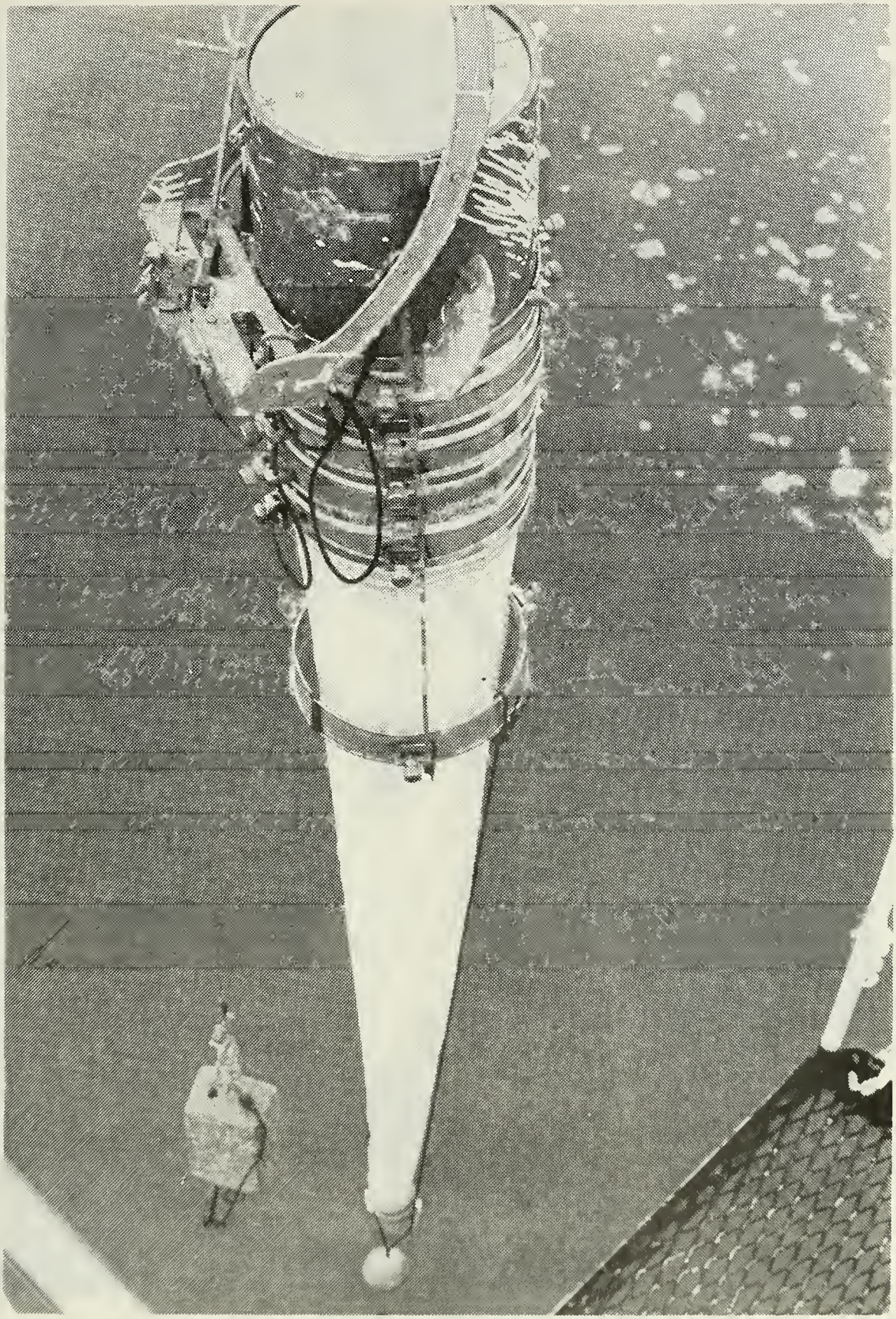


Figure 12(b). First generation net system on the Clarke-bumpus sampler attached to the hydrowire.

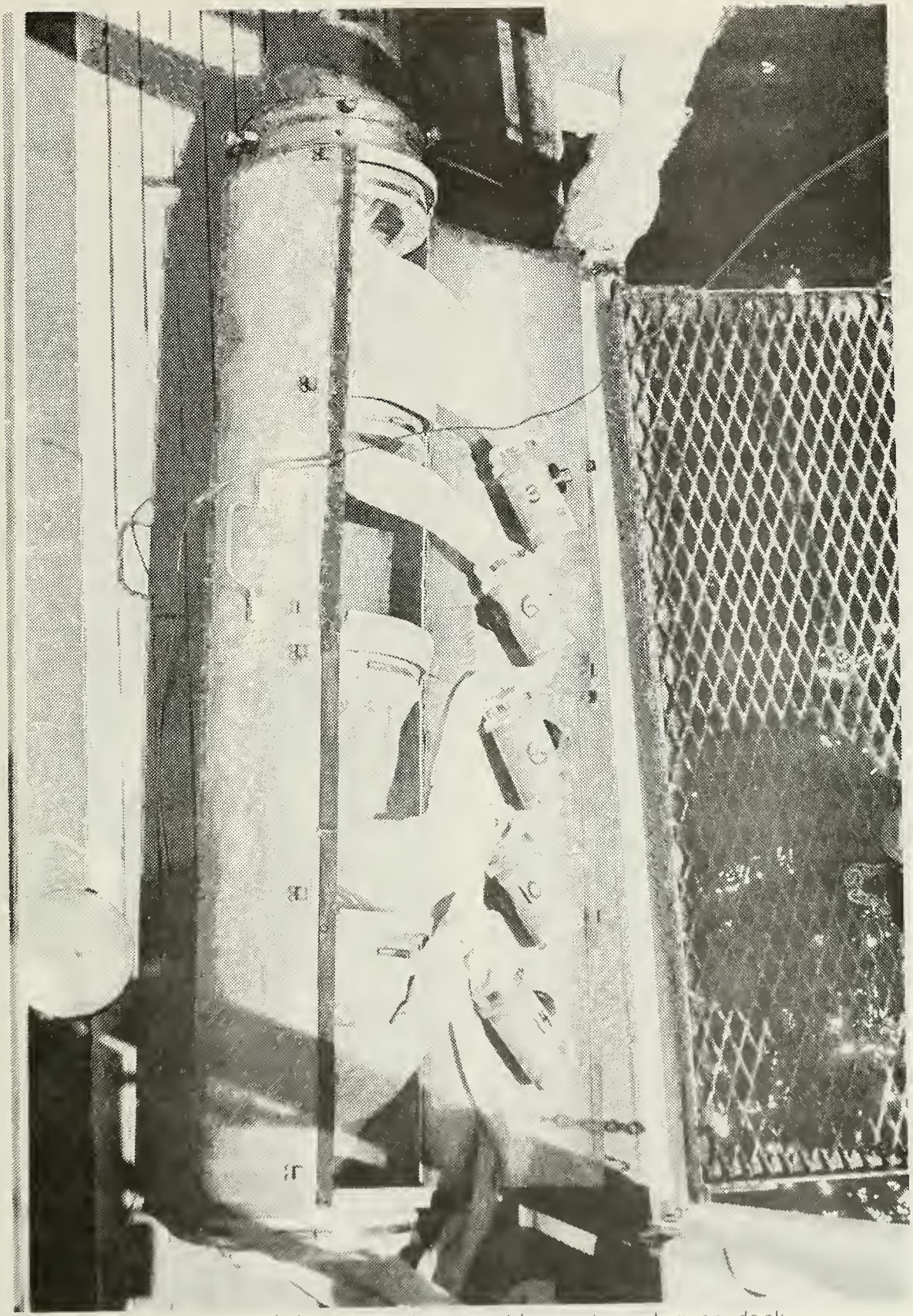


Figure 13(a). Second generation net system on deck with nets and buckets exposed

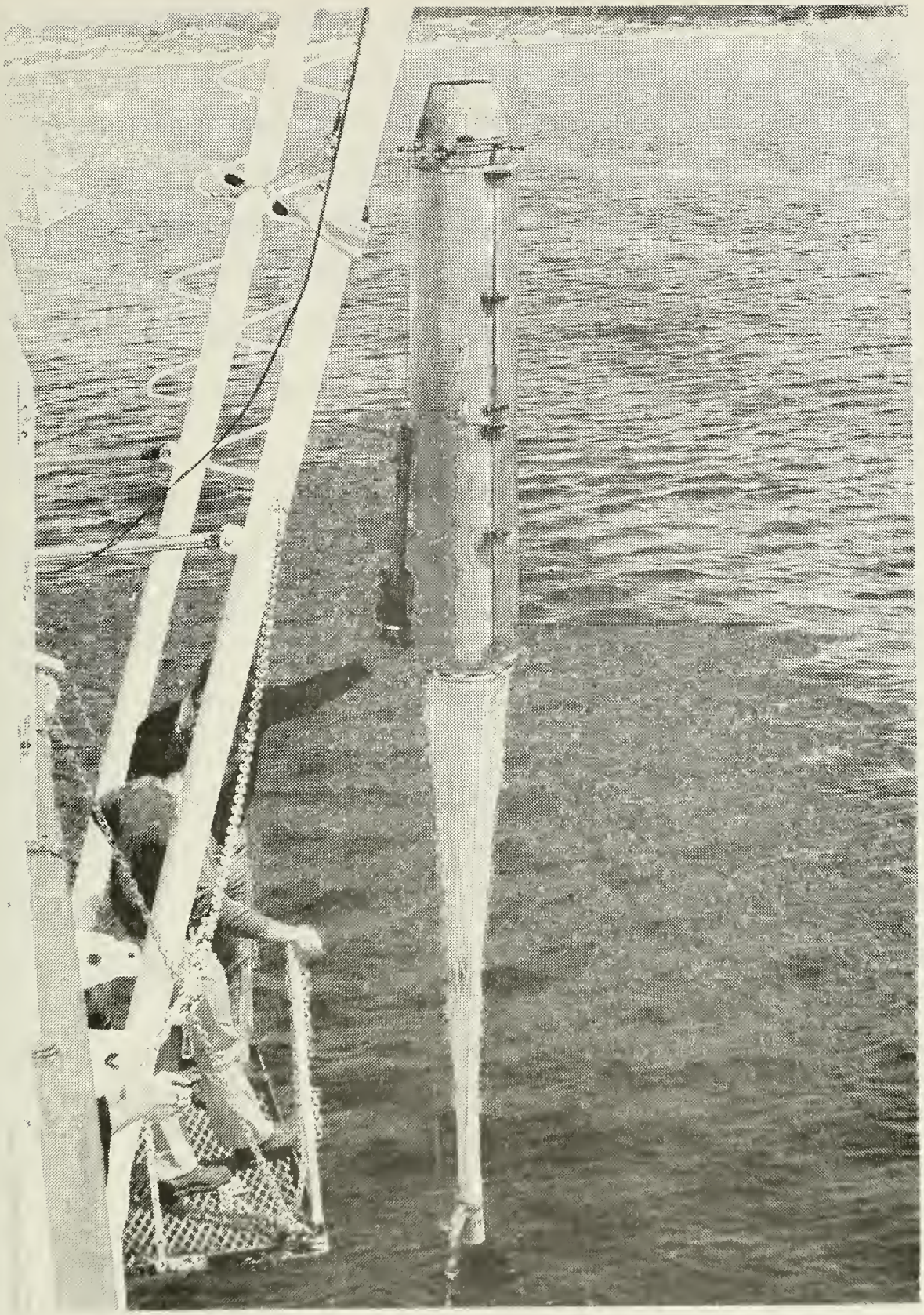


Figure 13(b) Second generation net system attached to the hydrowire.

taken while at sea. Nansen bottle casts were used to determine T-S diagrams and to collect nutrient samples for analyses with a "Technicon Autoanalyzer". Weather, sounding, and sea surface temperature data were also observed. A mechanical BT was attached to the second generation net system to determine the exact depth of the tow and to examine the temperature profile. The results of this data will be presented at a later date (Traganza, 1974).

IV. RESULTS

The experiments described above were conducted to determine the quantitative capability of estimation of zooplankton biomass by carbon analysis with the LECO Carbon Analyzer.

A. STANDARDIZATION

1. Benzoic Acid

The various weights (o - 50 mg) and corresponding DVM readings from each of the runs of benzoic acid are given in Table II. The apparatus blank values, which are included despite the smallness of their numbers, were considered to be part of the analysis procedure. Thus, by approximation of negative and near zero readings and of small weights, the analysis of copper and iron accelerators alone (called "blanks") contributed to the data taken and the subsequent results. Since benzoic acid has a known carbon content (68.8487%C), each of the weights of the standard were converted to carbon (mg).

The composite graph of benzoic acid data points for the DVM readings and weights was plotted by the method of "Best Fit" and is shown in Figure 14(a). The linear relationship and high correlation coefficient ($r = 0.9830$) of this graph indicates that the DVM reading was directly proportional to the weight of the benzoic acid sample. After conversion of all weights to carbon, the composite graph of DVM readings and corresponding carbon values were plotted using the method of "Best Fit," also (Figure 14(b). This plot represents a "standard curve" since the carbon content of any benzoic acid sample of unknown weight could be determined from it. This can be done by solving for

TABLE II

PLOTTED DATA FOR BENZOIC ACID CALIBRATION RUNS

Run #1

	Mass (mg)	DVM	Carbon (mg) (0.688487 x mass)
	24.5	1.111	16.87
	20.5	.946	14.11
	13.9	.660	9.570
	9.2	.464	6.334
	45.6	2.025	31.40
	27.4	1.330	18.86
	12.7	.687	8.744
	22.7	1.045	15.63
	8.2	.434	5.646
	22.3	.929	15.35
	15.9	.753	10.95
Blank	0.1	.012	.06885
Blank	0.1	.004	.06885
Blank	0.1	.003	.06885

Total number of data points on Run #1: n = 14

Run #2

Mass (mg)	DVM	Carbon (mg) (0.688487 x mass)
19.3	.722	13.29
24.5	1.380	16.87
29.0	1.210	19.97

	Mass (mg)	DVM	Carbon (mg) (0.688487 x mass)
	19.3	.722	13.29
	24.5	1.380	16.87
	29.0	1.210	19.97
	25.1	1.153	17.28
	13.2	.601	9.088
	18.1	.773	12.46
	19.0	1.069	13.08
	8.4	.431	5.783
	10.0	.595	6.885
	8.7	.489	5.990
	4.3	.248	2.960
	24.3	1.377	16.73
	32.7	1.343	22.51
Blank	0.1	.008	.06885
Blank	0.1	.003	.06885
Blank	0.1	.001	.06885

Total number of data points on Run #2: n = 16

Mass (mg)	DVM	Carbon (mg) (0.688487 x mass)
4.6	.250	3.167
6.1	.310	4.200
7.9	.409	5.439
10.6	.488	7.298
15.2	.723	10.47

Run #3 (con.)

	Mass (mg)	DVM	Carbon (mg) (0.688487 x mass)
	22.3	1.143	15.35
	30.0	1.219	20.65
	18.0	.814	12.39
	7.5	.390	5.164
	8.7	.449	5.990
	25.5	1.263	17.56
	12.8	.443	8.813
	13.6	.691	9.363
	15.0	.747	10.33
	15.0	.704	10.33
	10.7	.543	7.367
Blank	0.1	.001*	.06885
Blank	0.1	.001*	.06885
Blank	0.1	.001*	.06885

Total number of data points on Run #3: n = 19

*DVM of these blanks represent averaged small positive values <+.002

Note: Small mass of 0.1 mg was used with all blanks.

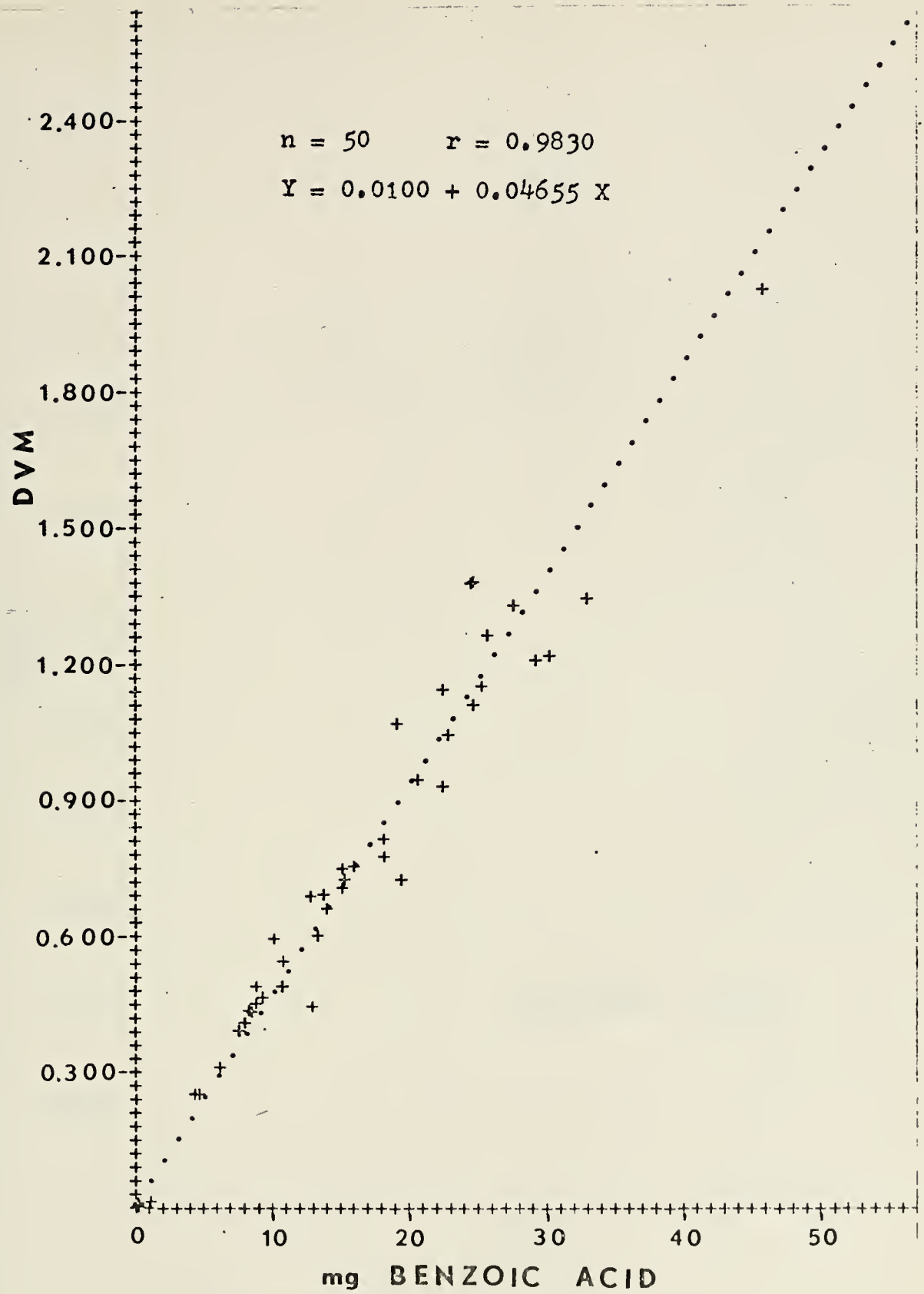


Figure 14(a). Composite graph of the three runs of benzoic acid.

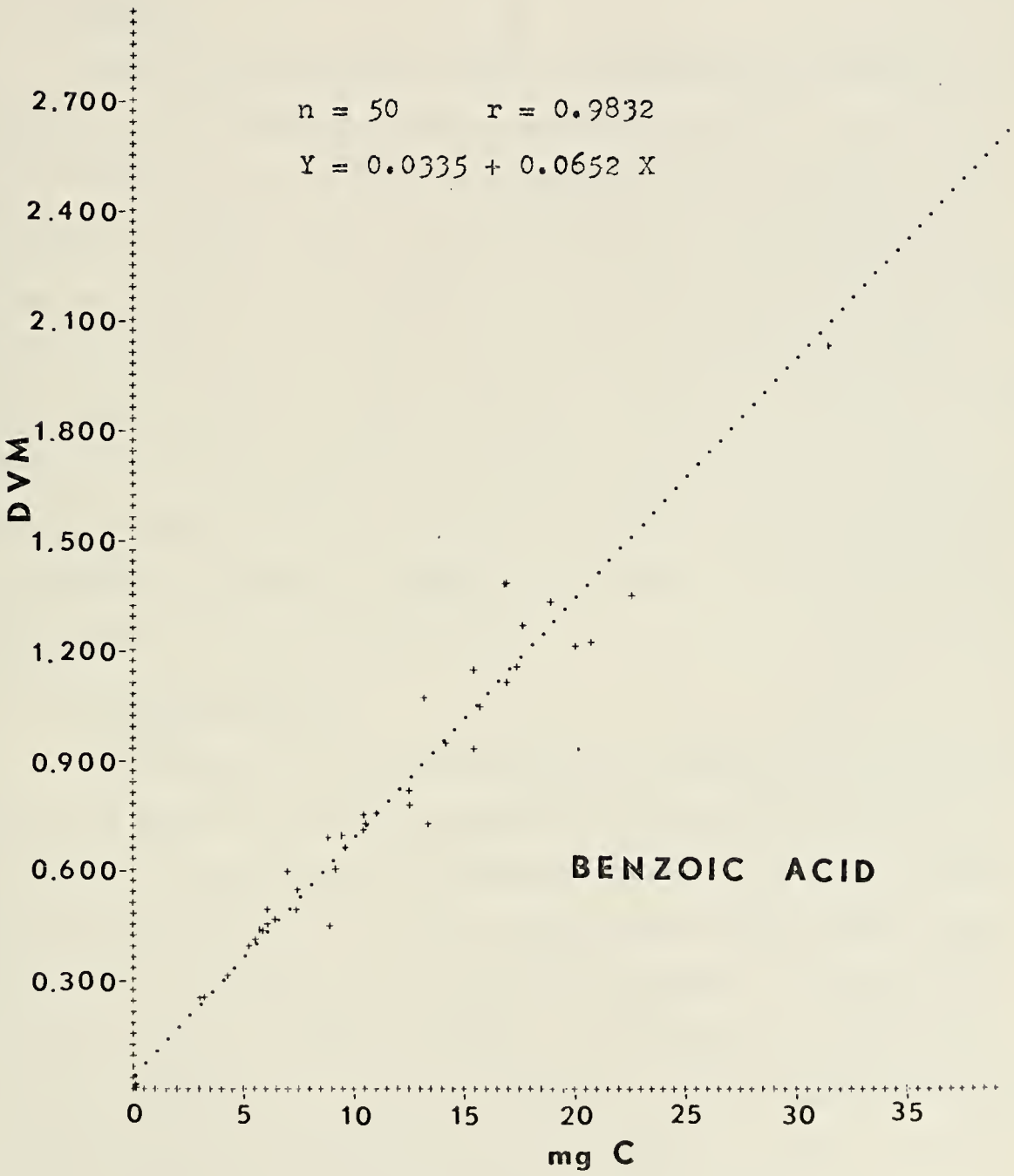


Figure 14(b). Standard curve for benzoic acid.

the carbon content, X, by using the equation of the standard curve and substituting the DVM reading for Y or by using the graph itself. The linear plot and high correlation coefficient (r = 0.9832) of the benzoic acid standard curve suggest a direct relationship between DVM and carbon.

2. Casein

The various weights (0-71 mg) and corresponding DVM values and weights, including apparatus blanks are given in Table III. As was the case in benzoic acid, the linear relationship and high correlation coefficient (r = 0.9802) of this graph demonstrate that the DVM reading was directly proportional to the weight of casein sample (Figure 15(a)). Note that the equation of this line is very different from that of the DVM versus sample weight for benzoic acid (Figure 14(a)). The "standard curve" for casein (53.13% C) was also plotted after conversion of each weight sampled to carbon. Again, a linear plot and high correlation coefficient (r = 0.9802) of the casein standard curve propose a direct relationship between DVM and carbon (Figure 15(b)).

3. Infrared Analysis

Interference with the DVM reading was evaluated by infrared analysis of the gaseous combustion products which entered the thermal conductivity cell in consideration of possible nitrogenous oxides. Two casein samples denoted in Table III, and one freeze-dried plankton sample (see Appendix D, Cruise 7403) were selected for evaluations. All three were potential sources of oxides of nitrogen in the combustion products since they contained protein, a source of nitrogen. Because temperature reached 1600^{o}C in the induction furnace during the combustion process, this reaction was a source of NO (nitric oxide). At the high temperatures, NO_2 was not formed rapidly enough to appear in the exhaust gases (Stoker and

TABLE III

PLOTTED DATA FOR CASEIN CALIBRATION RUNS

Run #1

	Mass (mg)	DVM	Carbon (mg) (0.5313 x mass)
	5.9 **	.290	3.135
	16.9	.490	8.979
	35.8	1.058	19.02
	71.0	2.532	37.72
	2.9	.146	1.541
	6.0	.296	3.188
	4.8	.209	2.550
	1.4	.069	.7438
	14.9	.590	7.916
	15.8	.578	8.395
	10.1 **	.377	5.366
	0.6	.027	.3188
	13.1	.429	6.960
	8.0	.258	4.250
	24.2	.702	12.86
	8.5	.335	4.516
	2.7	.115	1.435
Blank	0.1	.0001 *	.05313

Total number of data points on Run #1: n = 18

* DVM of these blanks represent small negative values.

**These samples were also used for Infrared analysis.

Run #2

	Mass (mg)	DVM	Carbon (mg) (0.5313 x mass)
	1.3	.062	.6907
	8.3	.385	4.410
	10.9	.496	5.791
	27.8	1.125	14.77
	22.6	.779	12.01
	12.1	.496	6.429
	9.7	.456	5.154
	16.5	.507	8.766
	9.5	.299	5.047
	6.8	.215	3.613
	5.7	.246	3.028
	17.7	.584	9.404
	25.0	.714	13.28
	18.7	.580	9.935
	30.4	.975	16.15
	20.1	.633	10.68
	42.6	1.501	22.63
Blank	0.1	.0001 *	.05313

Total number of data points on Run #2: n = 18

* DVM of these blanks represent small negative values.

Run #3

	Mass (mg)	DVM	Carbon (mg) (0.5313 x mass)
	0.9	.048	.4782
	2.6	.126	1.381
	8.4	.398	4.463
	24.6	1.173	13.07
	36.9	1.285	19.60
	8.3	.339	4.410
	17.7	.754	9.404
	11.1	.334	5.897
	13.4	.496	7.119
	33.7	1.287	17.90
	28.0	1.187	14.88
	20.7	.752	11.00
	13.9	.420	7.385
	7.7	.340	4.091
	4.4	.167	2.338
	10.2	.476	5.419
	12.5	.559	6.641
	15.5	.413	8.235
Blank	0.1	.0001*	.05313

Total number of data points on Run #3: n = 19

* DVM of these blanks represent small negative values.

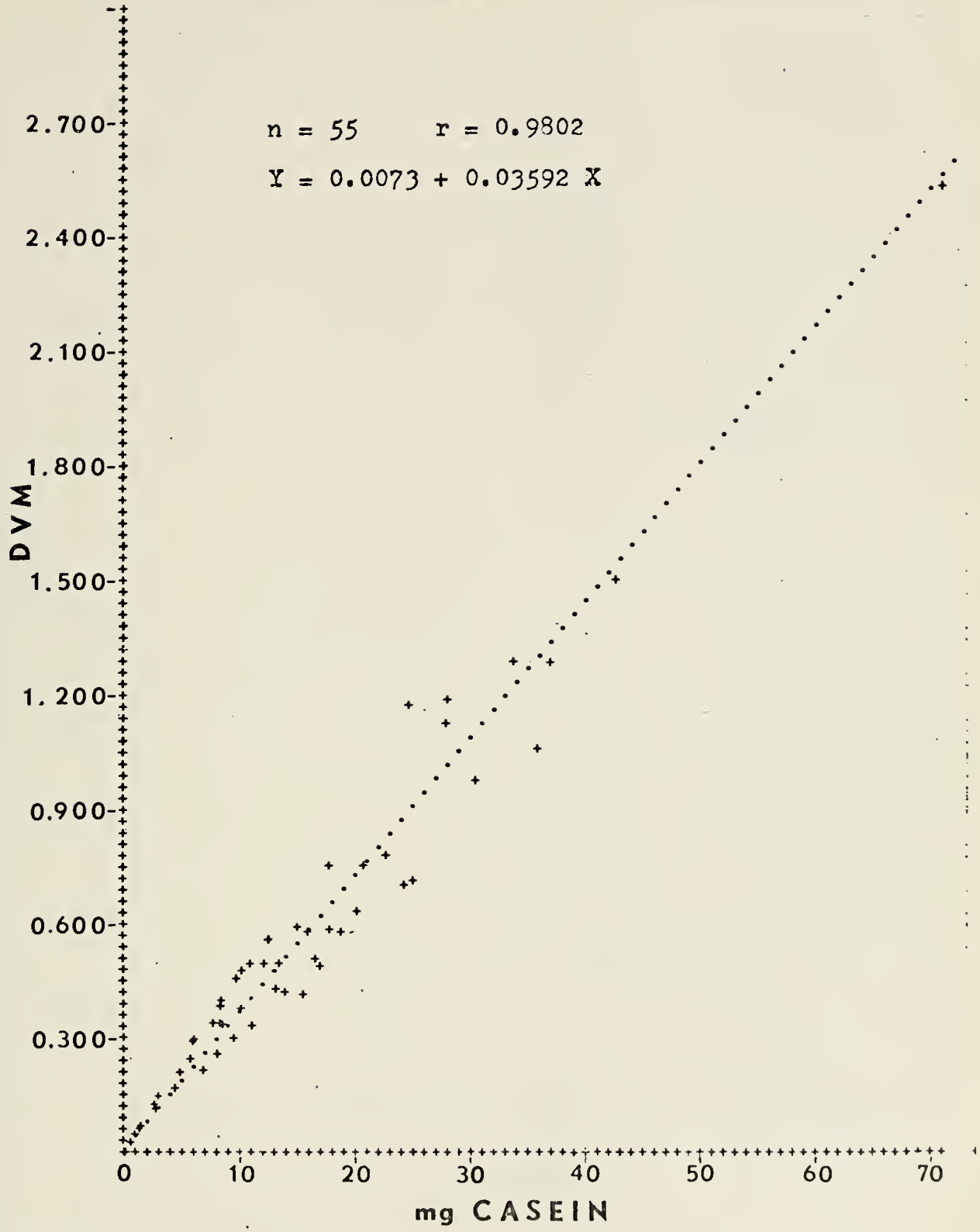


Figure 15(a). Composite graph of the three runs of casein.

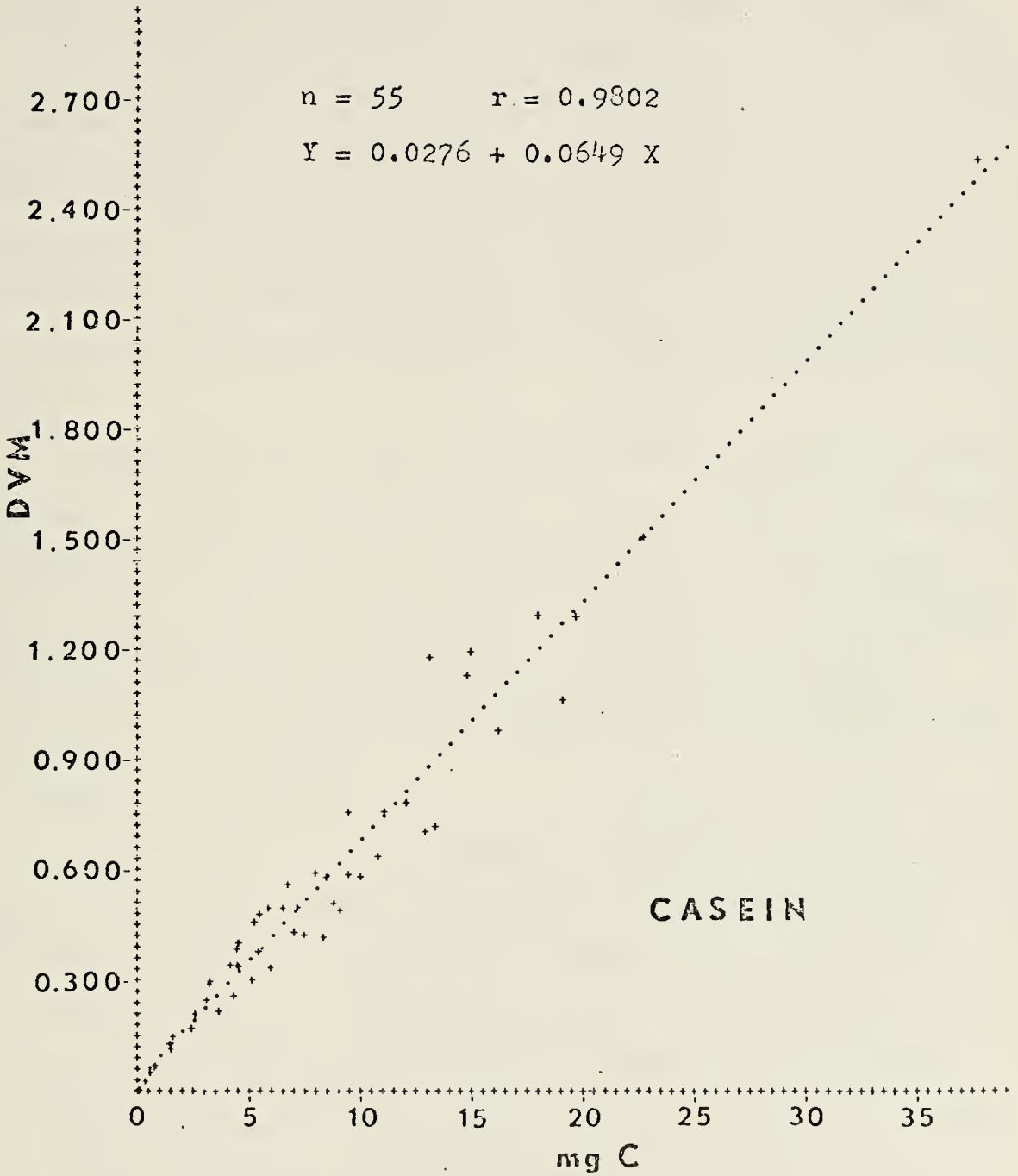


Figure 15(b). Standard curve for casein.

Seager, 1972). The presence of NO can be seen in all samples in the infrared spectra (Figures 16(a) and 17(a)) at a frequency of about 800 cm^{-1} (Pouchert, 1970). Even the blank and carrier gas (O_2) samples show a peak in the spectrum at a frequency near 800 cm^{-1}, indicating that some nitrogen entered the system at some time, *e.g.*, when the furnace was unloaded and loaded. By visual inspection the relative magnitudes of the NO peaks appear to be roughly equal and low compared to CO_2, which implies a constant amount of NO formation. Thus, due to the constancy of the NO peak, no biased results occurred from NO interference. No significant peaks occurred in the spectrum due to the presence of NO_2 as suspected (Pouchert, 1970).

The near-infrared spectra of Figures 16(b) and 17(b) show a strong CO_2 peak at a frequency of about 2349 cm^{-1} (Pouchert, 1970) in both casein samples and plankton sample. The carrier gas (O_2) and "blank" samples (accelerators only) do not have this peak as one might expect (Figure 23). A possible harmonic peak of CO_2 exists in the infrared spectrum near 1270 cm^{-1} (Dr. C. F. Rowell, personal communication) of the samples shown in Figures 16(a) and 17(a).

A final possibility for nitrogen was the presence of N_2O (nitrous oxide). This gas has a thermal conductivity at 27°C of 4.13 cal sec^{-1} $cm^{-1}deg^{-1}$ compared to 3.96 cal sec^{-1} cm^{-1} deg^{-1} for CO_2 (Ewing, 1972). Not only might this gas affect the DVM reading like other oxides of nitrogen, but its presence would affect the thermal conductivity sensor in very much the same way as CO_2. Only by infrared analysis or some other means could this be tested. All of the samples indicate that there is no noticeable N_2O peak (Pouchert, 1970) in the infrared spectrum and that there was an insignificant amount of interference from this gas.

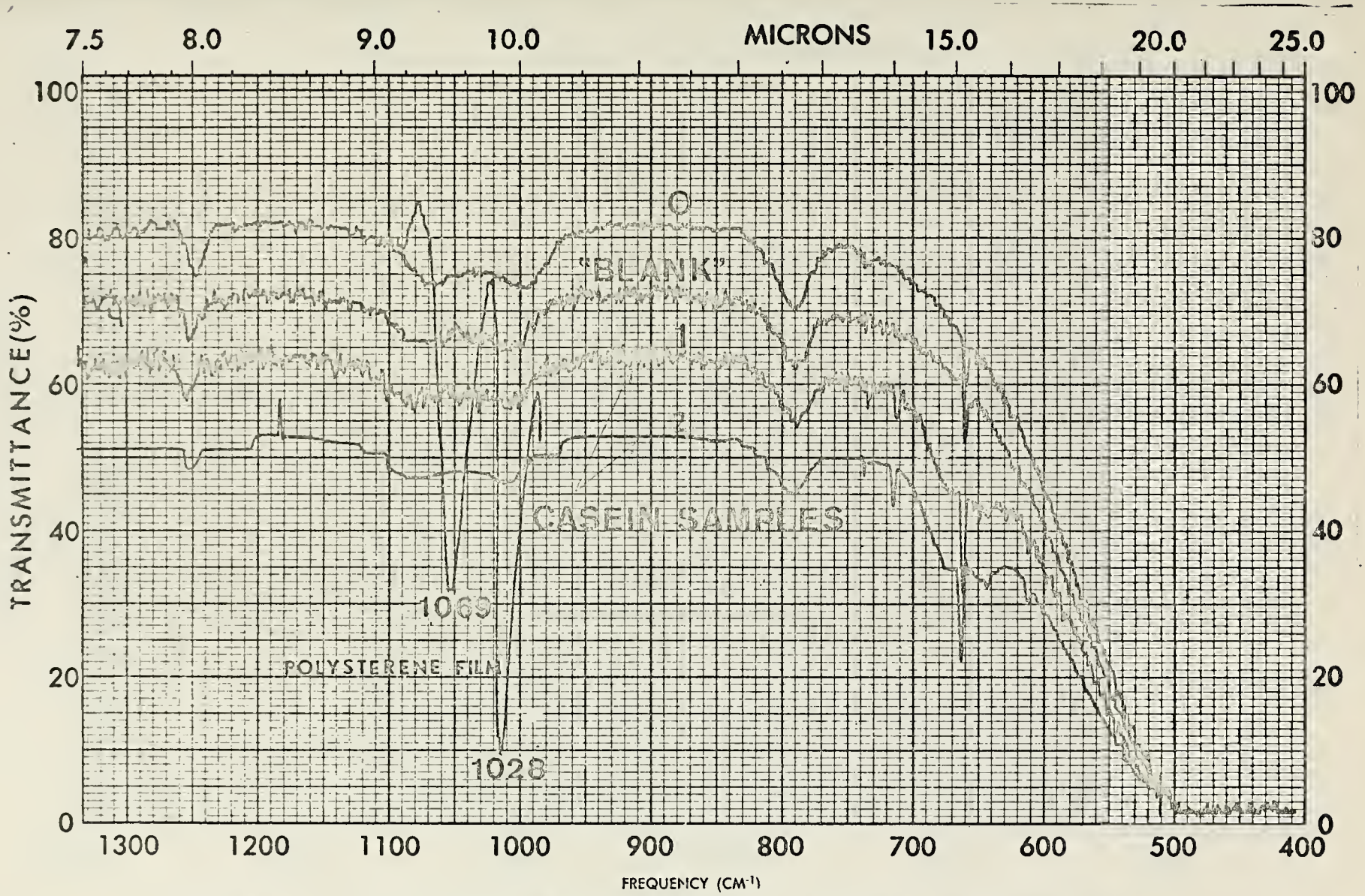


Figure 16(a). Infrared spectrum for carrier gas (O_2), "blank", and casein samples. The polystyrene film is used to calibrate the spectrum.

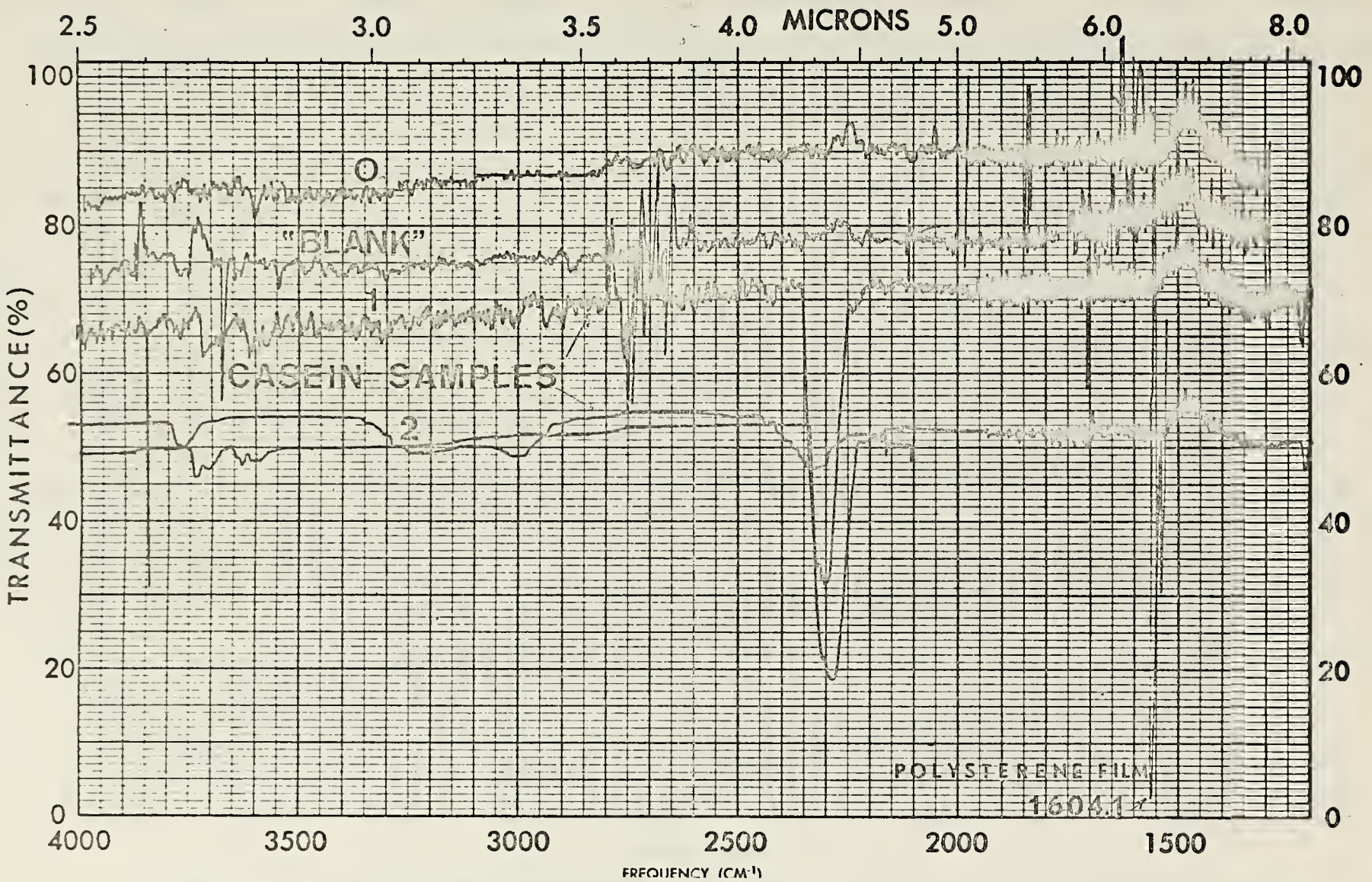


Figure 16(b). Near infrared spectrum for carrier gas (O_2), "blank", and casein samples. The polystyrene film is used to calibrate the spectrum.

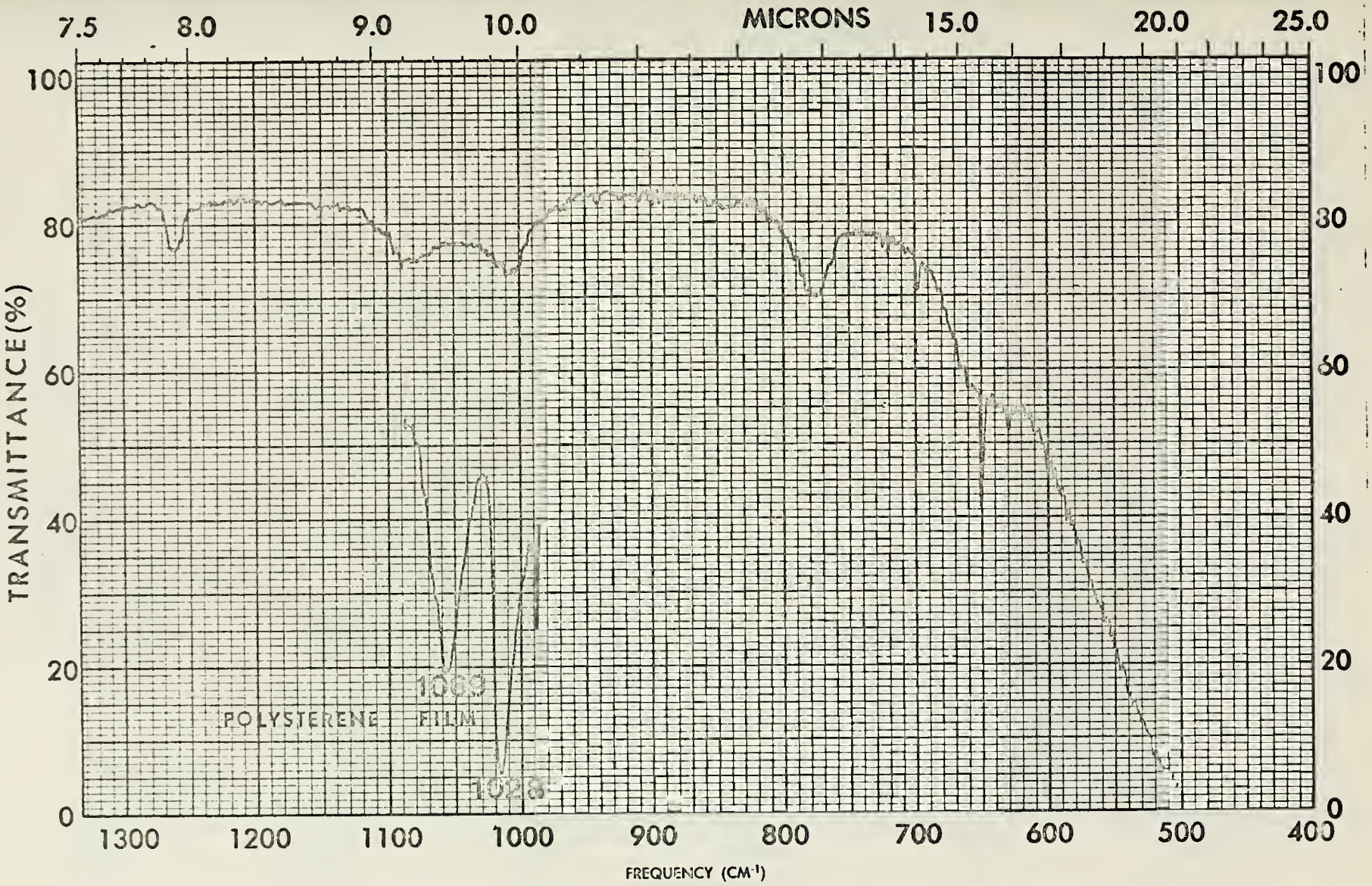


Figure 17(a). Infrared spectrum for plankton sample. The polystyrene film is used to calibrate the spectrum.

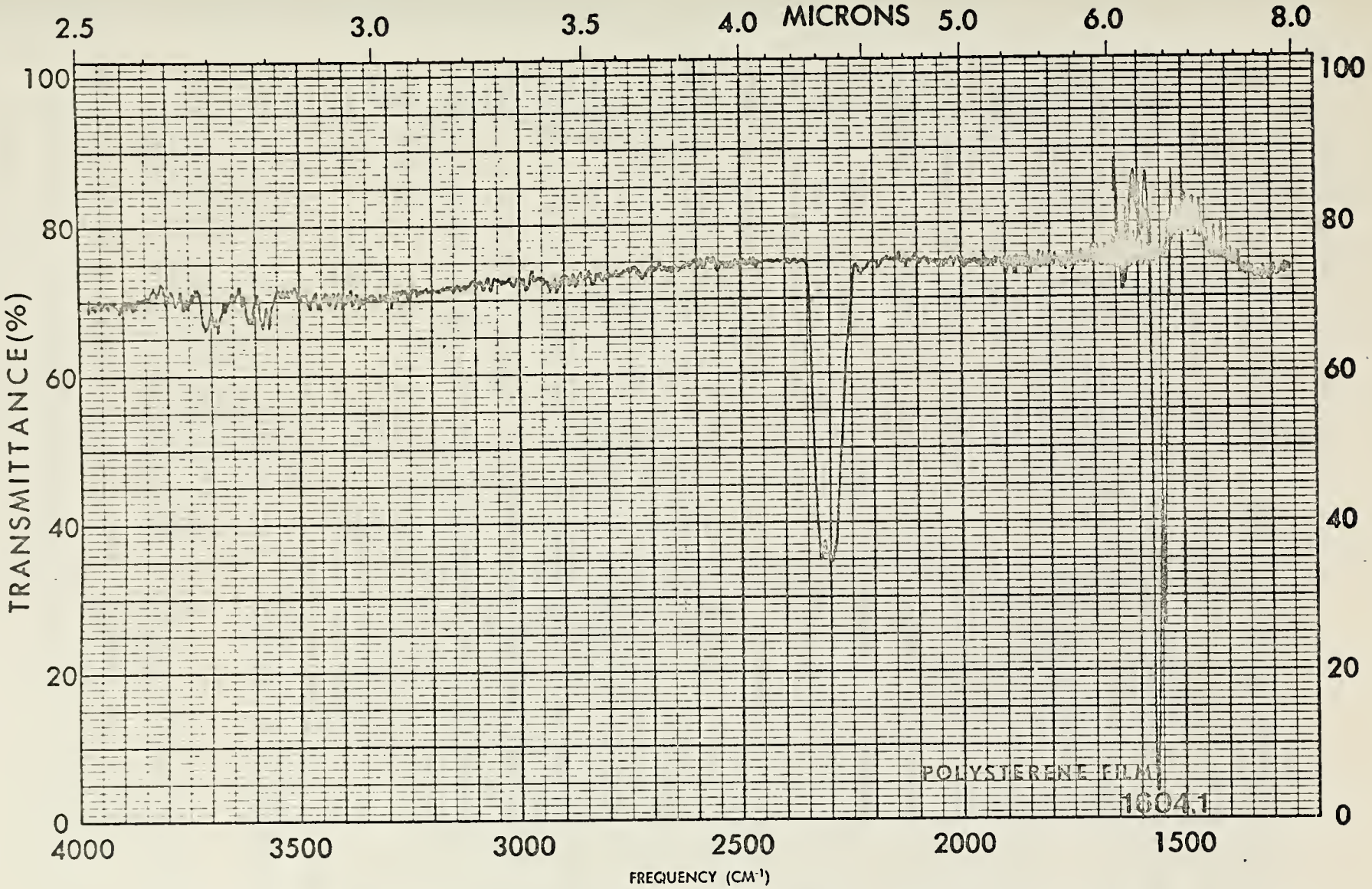


Figure 17(b). Near Infrared spectrum for plankton sample. The polystyrene film is used to calibrate the spectrum.

B. CARBON CONTENT IN *Tigriopus californicus*

Two different experiments were attempted to quantitatively determine the total carbon content of the test organism. In both sets of carbon analyses, the DVM readings were converted to carbon by using a combined standard curve based on benzoic acid and casein. Its particular use is further explained in the Discussion and Conclusions section.

Weights (0-20 mg) of freeze-dried *T. californicus* and corresponding DVM readings from both experiments are given in Table IV. In the first experiment, all samples were of one size fraction, (greater than 297 m) while in the second experiment several size fractions were used. The DVM readings and weights were plotted by the method of "Best Fit" and are shown in Figures 18(a) and (b). Blanks were included in this and all other graphs associated with the carbon content experiments. The linear relationships and high correlation coefficients ($r = 0.9903$ and $r = 0.9928$ respectively) of the first and second experiments indicate that the DVM reading was directly proportional to the weight of the sample. After conversion of all DVM readings to carbon (Table IV) two graphs of carbon and sample weight were plotted in the same manner for each experiment (Figures 19(a) and (b)). The linear relationships and high correlation coefficients ($r = 0.9893$ and $r = 0.9917$, respectively) of these graphs indicate a direct proportionality of the carbon to weight in *T. californicus*. The final "Best Fit" plot (Figure 20) is a combination of the carbon and sample weights from both experiments.

Table IV also shows the percentage of carbon to freeze-dried weight for each sample analyzed. A mean carbon percentage was computed to be 40.2% for the first experiment (excluding the very small sample of 0.3 mg which gave an abnormally high DVM reading) and 37.8% for the second

TABLE IV

PERCENT CARBON DETERMINATION FOR *TIGRIOPUS CALIFORNICUS*

Experiment 1

Fraction Size	Mass (mg)	DVM	Carbon (mg)	%C (C/mass x 100)
>297 µm	0.3	.019	.2808	93.60
>297	1.2	.042	.6208	51.73
>297	0.6	.011	.1626	27.10
>297	0.3	.001	.0148	49.33
>297	1.5	.042	.6208	41.39
>297	1.1	.026	.3843	34.94
>297	3.0	.068	1.005	33.50
>297	2.4	.040	.5912	24.63
>297	5.6	.167	2.468	44.07
>297	5.2	.164	2.424	46.61
>297	14.3	.389	5.750	40.21
>297	10.3	.340	5.026	48.90
Blank	0.1*	.001		
Blank	0.1	.0001		

n = 14 for DVM vs. mass

N = 12 for C(mg) vs. mass (mg)

* Blanks given represent a blank of 0.000

Experiment 2

Fraction size	Mass (mg)	DVM	Carbon (mg)	%C (C/mass x 100)
>420 μm	12.9	.292	4.318	33.47
>420	6.6	.143	2.114	32.03
420 - 320	3.5	.089	1.316	37.60
420 - 320	8.1	.192	2.840	35.06
420 - 320	11.3	.298	4.407	39.00
320 - 297	2.0	.055	.8135	40.68
320 - 297	11.9	.298	4.407	37.03
320 - 297	3.8	.098	1.449	38.13
297 - 250	0.6	.027	.3993	66.55
297 - 250	0.2	.007	.1035	51.75
>420	28.7	.708	10.47	36.48
>420	8.9	.222	3.283	36.89
>420	10.1	.252	3.727	36.90
>420	9.7	.242	3.579	36.90
>420	12.2	.332	4.910	40.25
420 - 320	11.3	.332	4.910	43.45
420 - 320	14.0	.354	5.236	37.40
420 - 320	16.0	.375	5.546	34.66
320 - 297	9.4	.215	3.179	33.82
320 - 297	11.5	.252	3.727	32.41
320 - 297	21.0	.496	7.336	34.93
297 - 250	5.0	.101	1.494	29.88

Fraction size	Mass (mg)	DVM	Carbon (mg)	%C (C/mass x 100)
297 - 250 m	3.8	.084	1.242	32.68
297 - 250	8.6	.176	2.603	30.27
Blank	0.1	.0001		
Blank	0.1	.012		

n = 26 DVM vs. mass

N = 24 C vs. mass

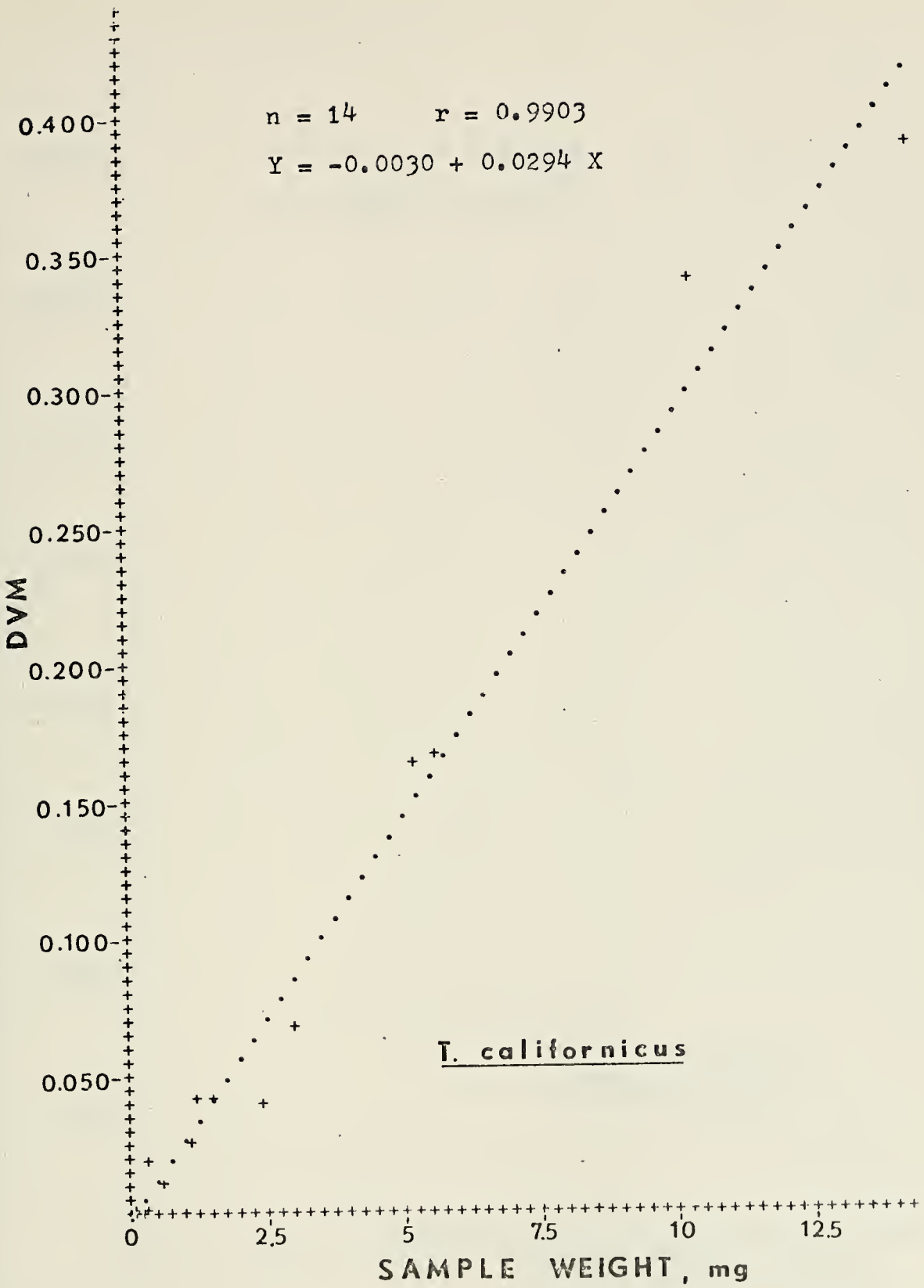


Figure 18(a). First experiment.

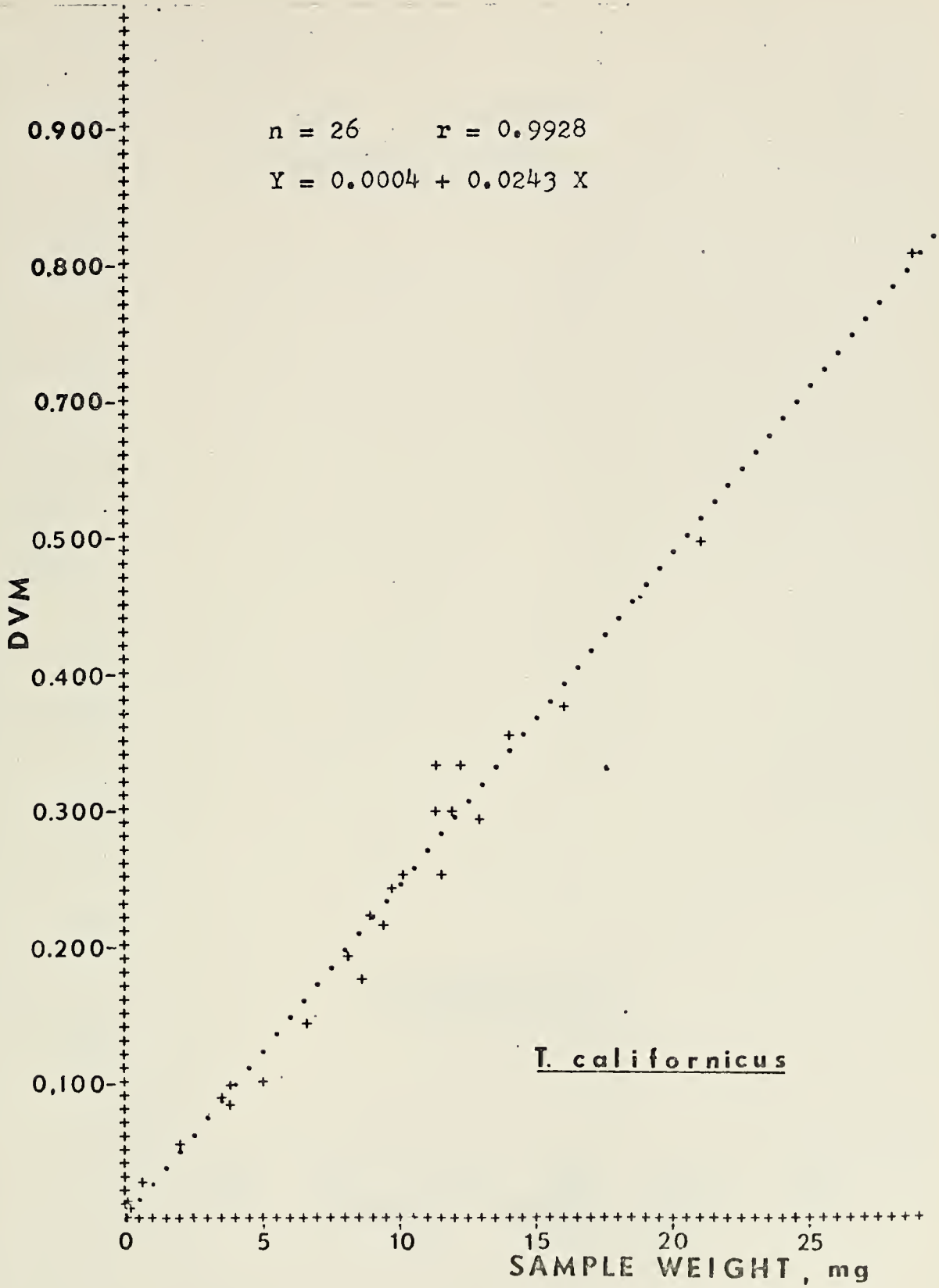


Figure 18(b). Second experiment.

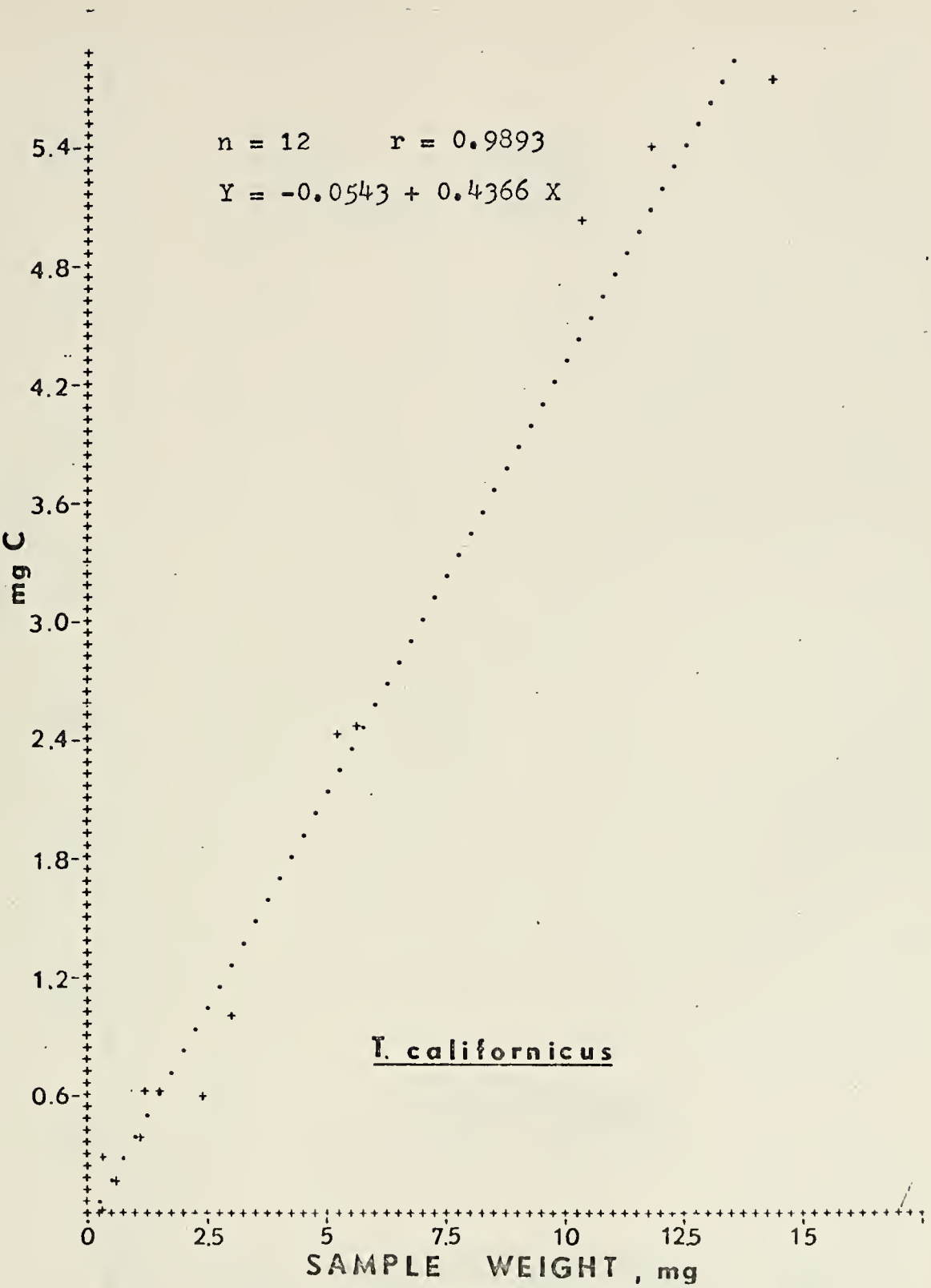


Figure 19(a). First experiment, % carbon determination

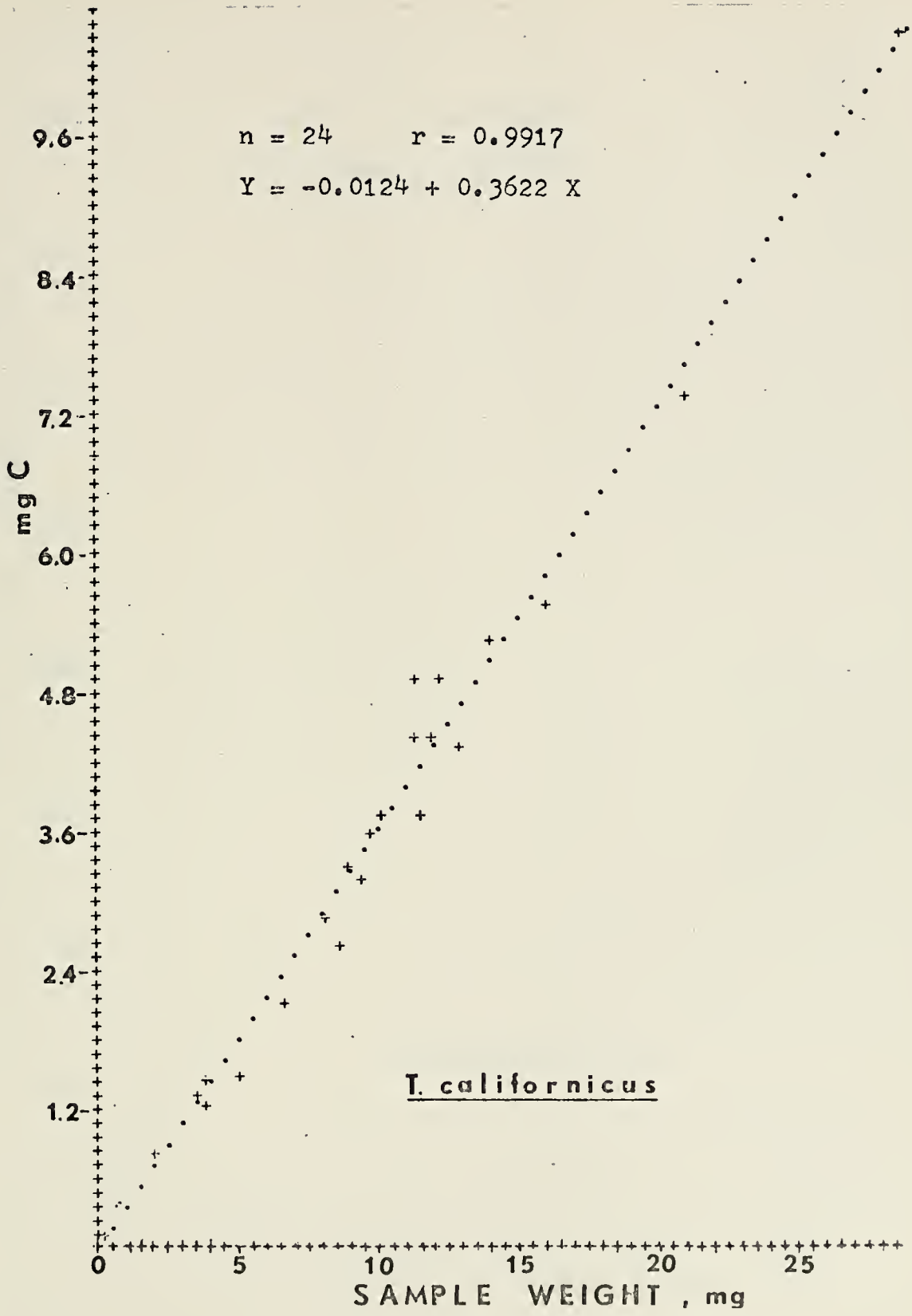


Figure 19(b). Second experiment, % carbon determination.

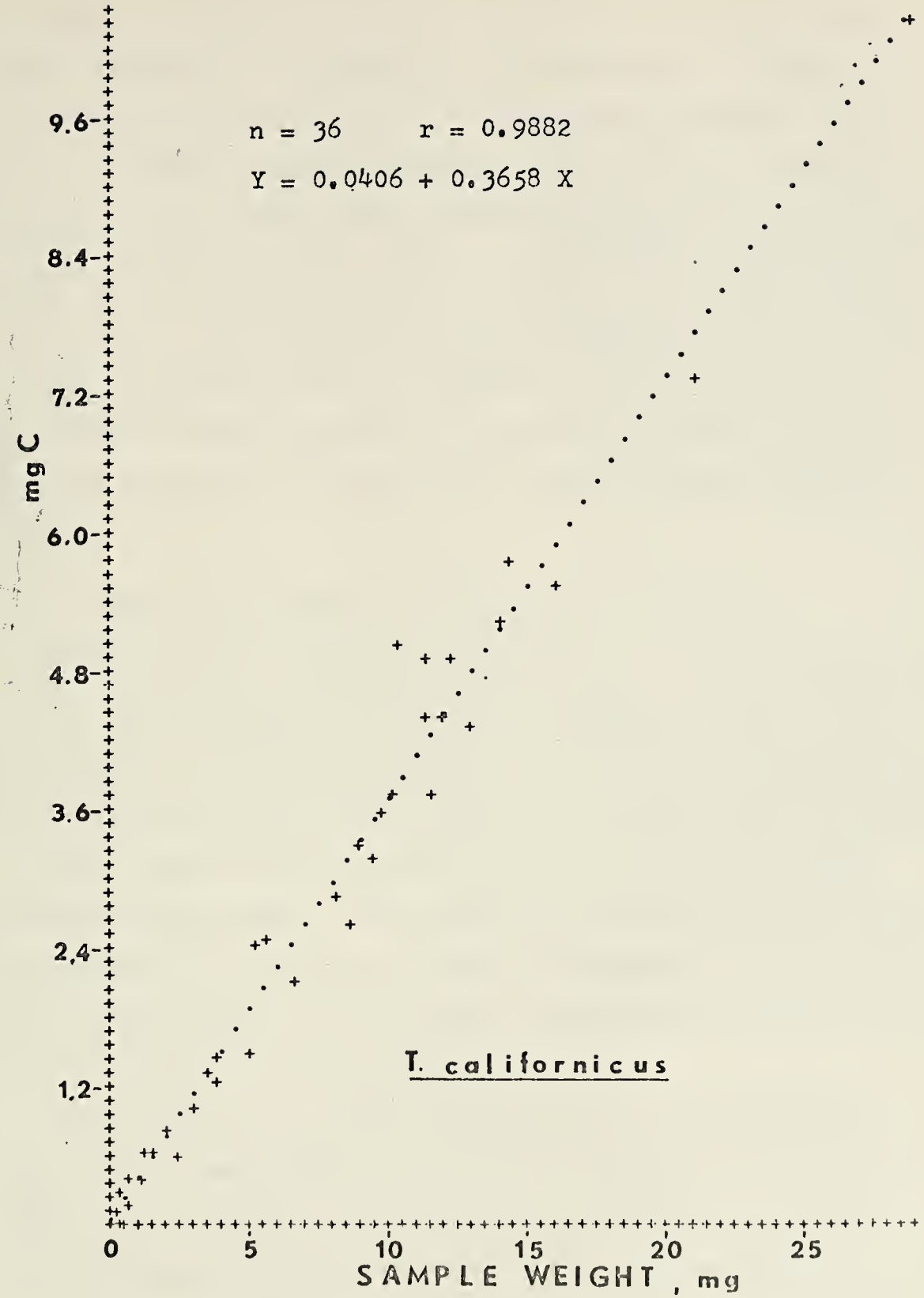


Figure 20. Combined data plot from both experiments.

experiment. No significant deviations were found in the carbon percentages of different size fractions in the second experiment. Finally, a combined mean carbon percentage of both experiments was computed to be 38.6%. Curl (1962) determined the total and organic carbon in terms of percent dry weight in "mixed copepods" to be 35.6% and in *Calanus finmarchicus*, a well-known pelagic copepod, to be 39.8%. The inorganic carbon in all but one of the 19 species and mixed collections of marine organisms that Curl analyzed was found to be negligible. Assuming that no significant inorganic carbon was present in *Tigriopus californicus*, the value of 38.6% carbon agrees well with Curl's data of total and organic carbon in copepods.

C. ATP-CARBON TO TOTAL CARBON RATIO IN *Tigriopus californicus*

The ATP-C to total C ratio was examined in order to determine the ratio of ATP to cell carbon and its constancy by different size groups in the test organism. The organisms analyzed for ATP and carbon were assumed to be alive (dead and detrital particulate matter passed through the screens).

Table V shows the ATP-C and carbon data from the first and second experiments, respectively. Also noted are the corrections applied and the size fractions of *T. californicus* used in the experiments. The "Best Fit" plots of ATP-C against total C are given in Figures 21(a) and (b) for the first and second experiments, respectively.

A third graph was done of the combined data from both experiments (Figure 21(c)). The 297-250 μm fraction was excluded since it was not common to both experiments and may have contained detritus. No significant differences were observed in the ATP-C total C ratio (Table V) between different size fractions. The linear relationship and correlation coefficient ($r = 0.9505$) of the composite plot suggests a constant ATP-C

TABLE V

ATP CARBON TO CARBON RATIO IN *TIGRIOPUS CALIFORNICUS*

Experiment 1

Fraction size (μm)	Mass (mg) Approximate	DVM	Carbon (mg) Uncorrected	Extraction volume Correction (mg)
> 420	6.2	.292	4.319	.2273
> 420	8.0	.230	3.400	.1889
420 - 297	7.3	.275	4.067	.2113
420 - 297	12.0	.487*	7.203	.3742
420 - 297	8.0	.218	3.222	.1718
Control	.5	.011	.163	

Before Filtration

C_L (mg)	ATP-C (mg)	ATP-C/C_L x100
4.392	.0095	.2163
3.437	.0107	.3114
4.127	.0118	.2859
7.432	.0177	.2382
3.239	.0083	.2562

After Filtration

C_L (mg)	ATP-C (mg)	ATP-C/C_L x100
4.393	.0108	.2458
3.436	.0100	.2910
4.125	.0097	.2352
7.431	.0172	.2315
3.239	.0083	.2562

Three corrections applied to DVM derived carbon:

1. Volume extraction correction (added)
2. ATP-Carbon in ATP analysis (added)
3. Control contributed carbon (subtracted)

* Question in data - DVM is either .287 or .487.

Experiment 2

Run 1

Fraction Size(m)	Mass (mg) Approximate	DVM	Carbon (mg) Uncorrected	C_L (mg)	ATP-C (mg)	Ratio
>420	10.9	.384	5.679	5.636	.0158	.2803
>420	19.5	.387	5.724	5.679	.0144	.2536
420-297	23.6	.388	5.739	5.695	.0154	.2704
420-297	23.5	.357	5.280	5.235	.0143	.2732
297-250	12.4	.119	1.760	1.707	.0060	.3515
Control	0.1	.004	.059			

Run 2

>420	15.3	.311	4.660	4.556	.0138	.3029
>420	10.5	.141	2.086	1.973	.0060	.3041
420-297	17.8	.325	4.807	4.701	.0115	.2445
420-297	18.1	.468	6.922	6.821	.0166	.2434
297-250	15.8	.073	1.080	.9643	.0023	.2385
Control	1.5	.008	.118			

Two corrections applied to DVM derived carbon:

(1) Control contributed carbon (subtracted).

(2) ATP-Carbon in ATP analysis (added).

There was no volume extraction correction.

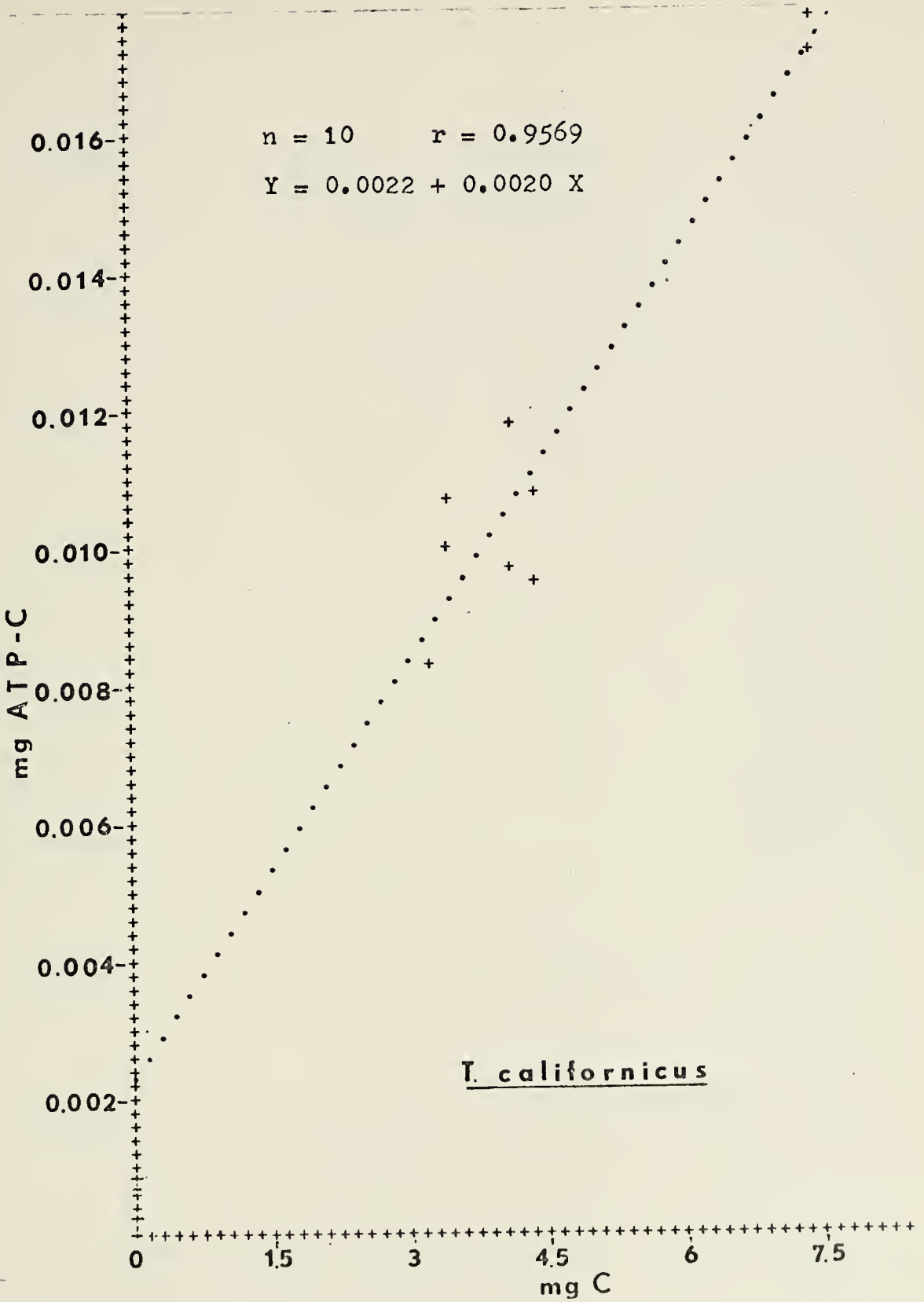


Figure 21(a). First experiment, ATP-C to total C ratio.

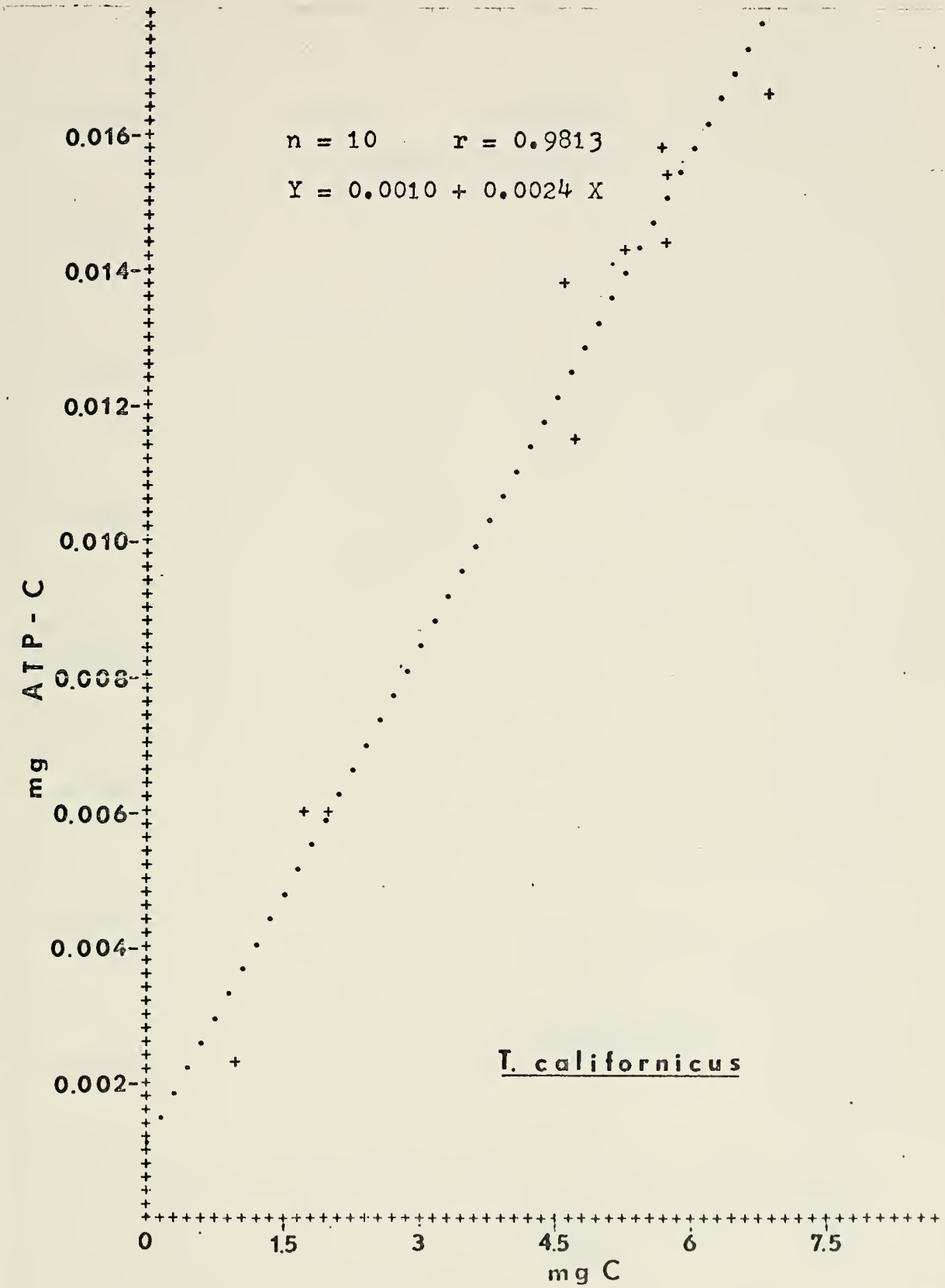


Figure 21(b). Second experiment; ATP-C to total C ratio.

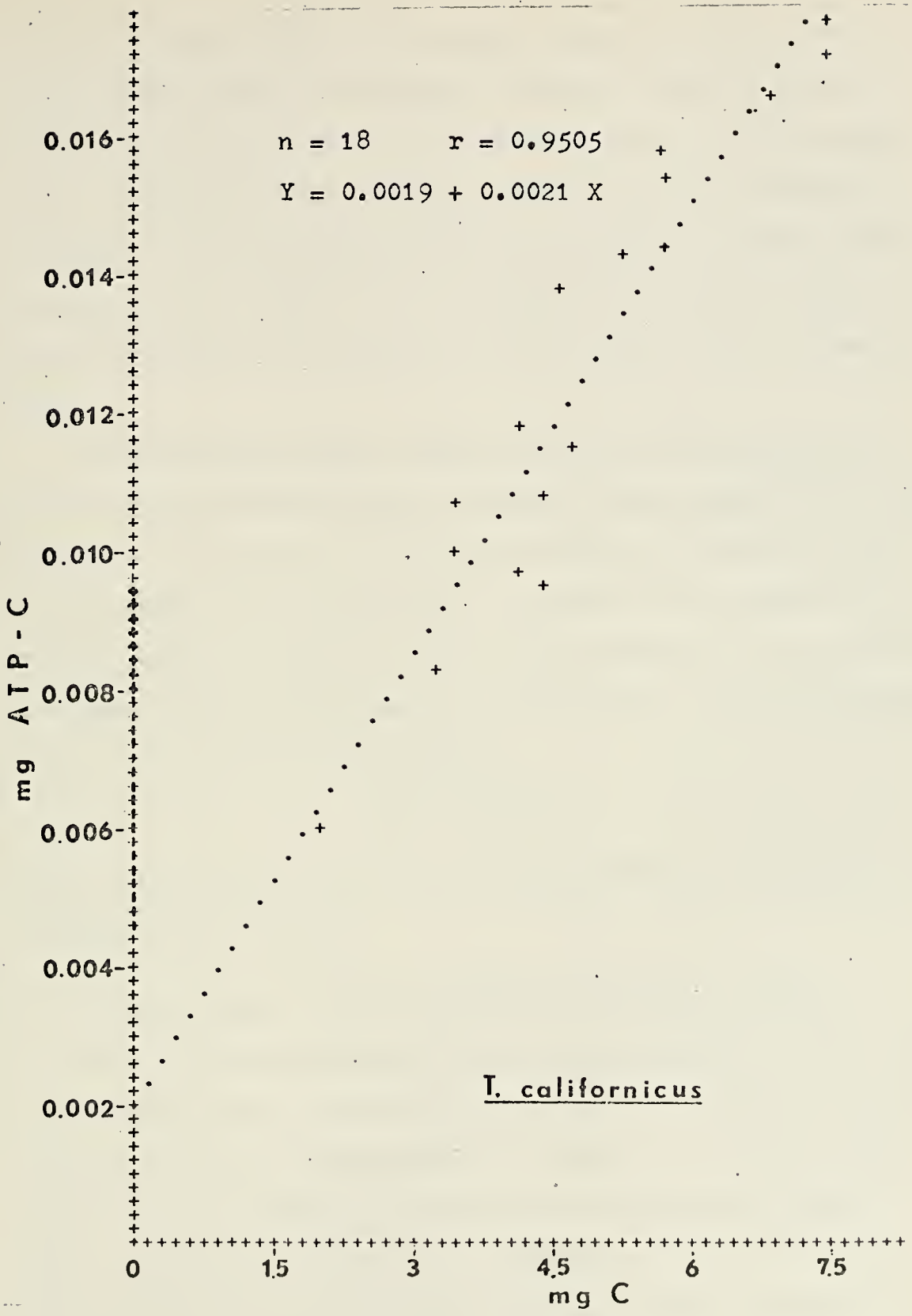


Figure 21(c). Combined data plot of both experiments, ATP-C to total C ratio.

to total C ratio in both size fractions. A mean percentage of ATP-C to total C from the data of the graph yielded 0.26%. Balch (1973) has reported a mean of 0.89% for the ATP-C to total C ratio, 0.78% for total carbon, and 1.89% for non-lipid body carbon, in *Calanus finmarchicus*. Holm-Hansen (1970) determined that "during exponential growth in batch culture, cellular contents of ATP remained at fairly uniform levels in unicellular algae and averaged 0.35% of the cellular organic carbon content."

From the assumption that analyses were made on live organisms, the total carbon determinations were considered to be a measure of the carbon present in living cells, *i.e.*, "living carbon" or C_L. Thus, the ratio can be expressed such that ATP-C/C_L = R, where R is the ratio and C_L is "living carbon". Since this mean ratio was determined for *T. californicus* as 0.2634×10^{-2}, ATP measurements converted to ATP-C can be combined with this ratio to solve for living carbon:

$$C_L = 1/R(\text{ATP-C})$$

which is approximately 380(ATP-C). All determinations of living carbon stemmed from this equation.

D. ATP-C AND CARBON ANALYSES IN ASSOCIATION WITH FIELD STUDIES

ATP and carbon analyses were made on "net zooplankton" samples from three cruises taken in the Monterey upwelling area (Appendix D). These analyses were used to compute total (C), living (C_L), and dead carbon ($C_D = C - C_L$), in each of the five net fractions for each station. (#3 = > 333 μm; #6 = 333-243 μm; #8 = 243-202 μm; #10 = 202-160 μm; #14 = 160-102 μm). The values were computed in terms of surface area (per square meter). The nose cone used in both net systems sampled a 0.041 m^2 column (0-200 m) of ocean and the resulting determinations were

converted to a square meter. Total carbon determinations on the "net zooplankton" samples were made using the LECO Carbon Analyzer. The use of ATP measurements and the ATP-C to carbon ratio in *T. californicus* was employed to compute living carbon estimates. The dead carbon estimates were computed by taking the difference between total and living carbon. Again, based on Curl (1962) the inorganic carbon in the marine organisms was presumed to be negligible. Thus, total particulate analysis of the "net zooplankton" samples represented total organic carbon.

Time series plots of total, living, and dead carbon for all cruises are given in Figures 22(a), (b), and (c), respectively. Night and day stations are indicated. Trend lines are also shown by connecting night and day mean values for each net fraction for each cruise. No adjustments were made to allow for minor errors in the data indicated in Appendix D. The trend lines shown in the summary plots indicate that there was an increase in biomass and in total, living and dead carbon mean estimates in each of the net fractions collected on the cruises from May to July, followed by a slight decrease in early August. Two net fractions provided exceptions to this trend, since #8 (243-202 μm) and #10 (202-160 μm) net fractions remained relatively constant. Living (C_L) and dead (C_D) carbon in fractions #6, #8, #10 and #14 reached a maximum of about 200 mg/m^2m whereas the highest value for any station for total carbon (*i.e.*, the sum of living plus dead (C)) was about 300 mg/m^2. The unbounded upper fraction (#3) contained up to 2000 mg/m^2 of total carbon and up to 800 mg/m^2 for living and 1600 mg/m^2 for dead carbon. Significant differences in night and day stations of each cruise occurred, *e.g.*, the difference was greater than 50% of the mean C_L (day) in the #6 fraction in July (see Discussion and Conclusions). Since the size fractions were only approximately 50 μm, a skewed distribution of biomass occurred in the net

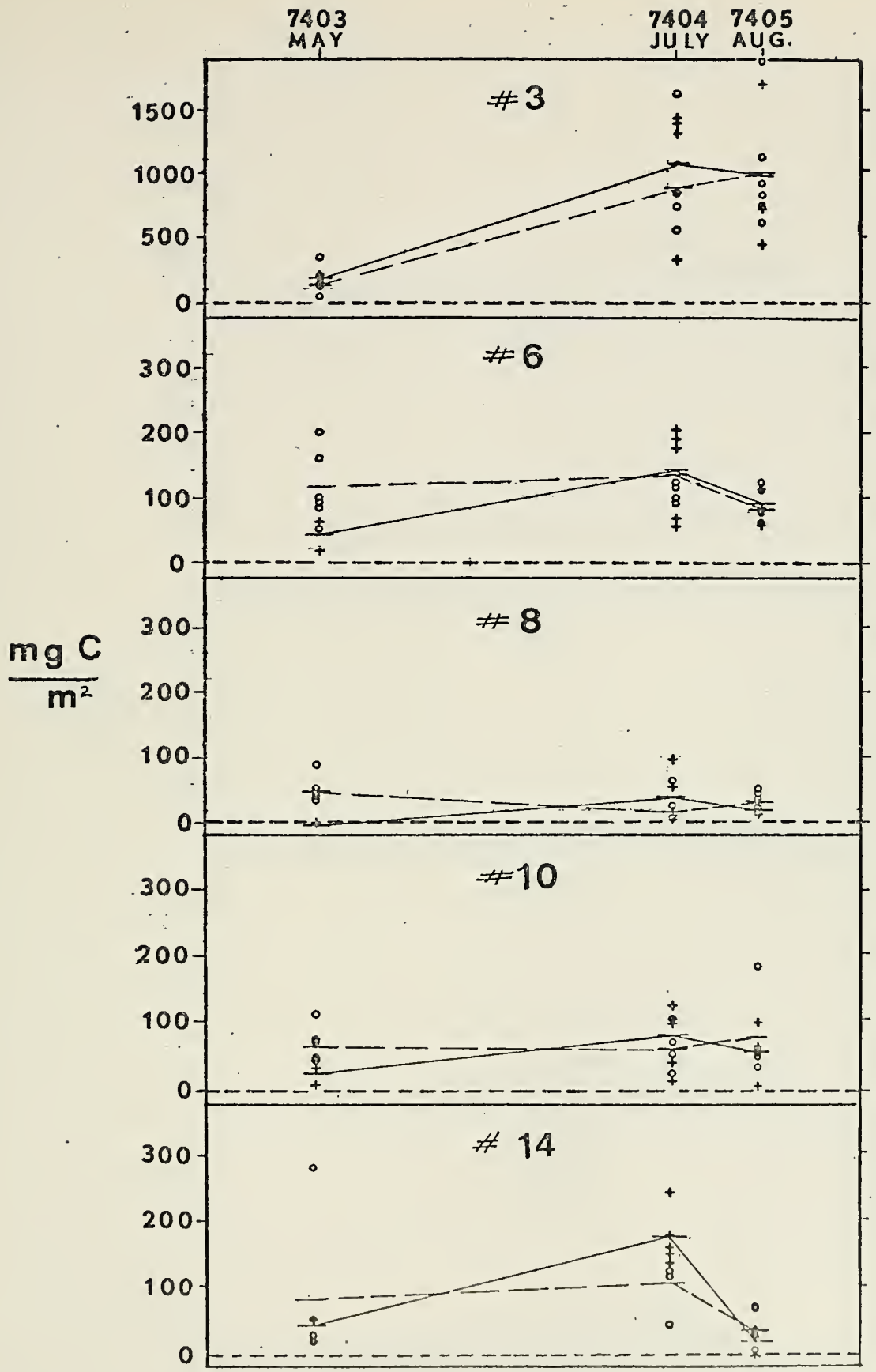


Figure 22(a). Total carbon (C) per surface area. + = night station, o = day station; — = night, --- = day

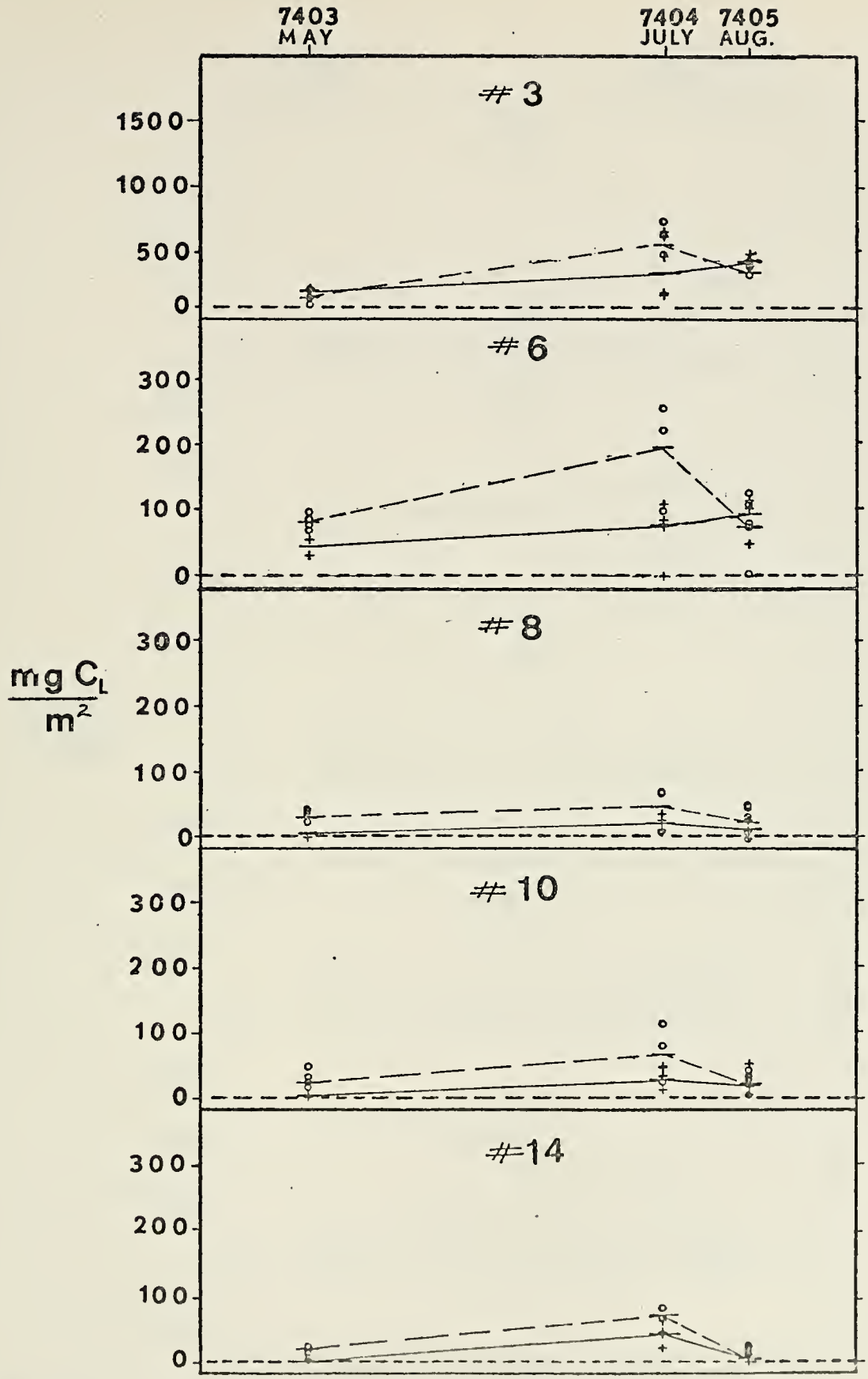


Figure 22(b). Living carbon (C_L) per surface area. + = night station, o = day station; ___ = night, --- = day.

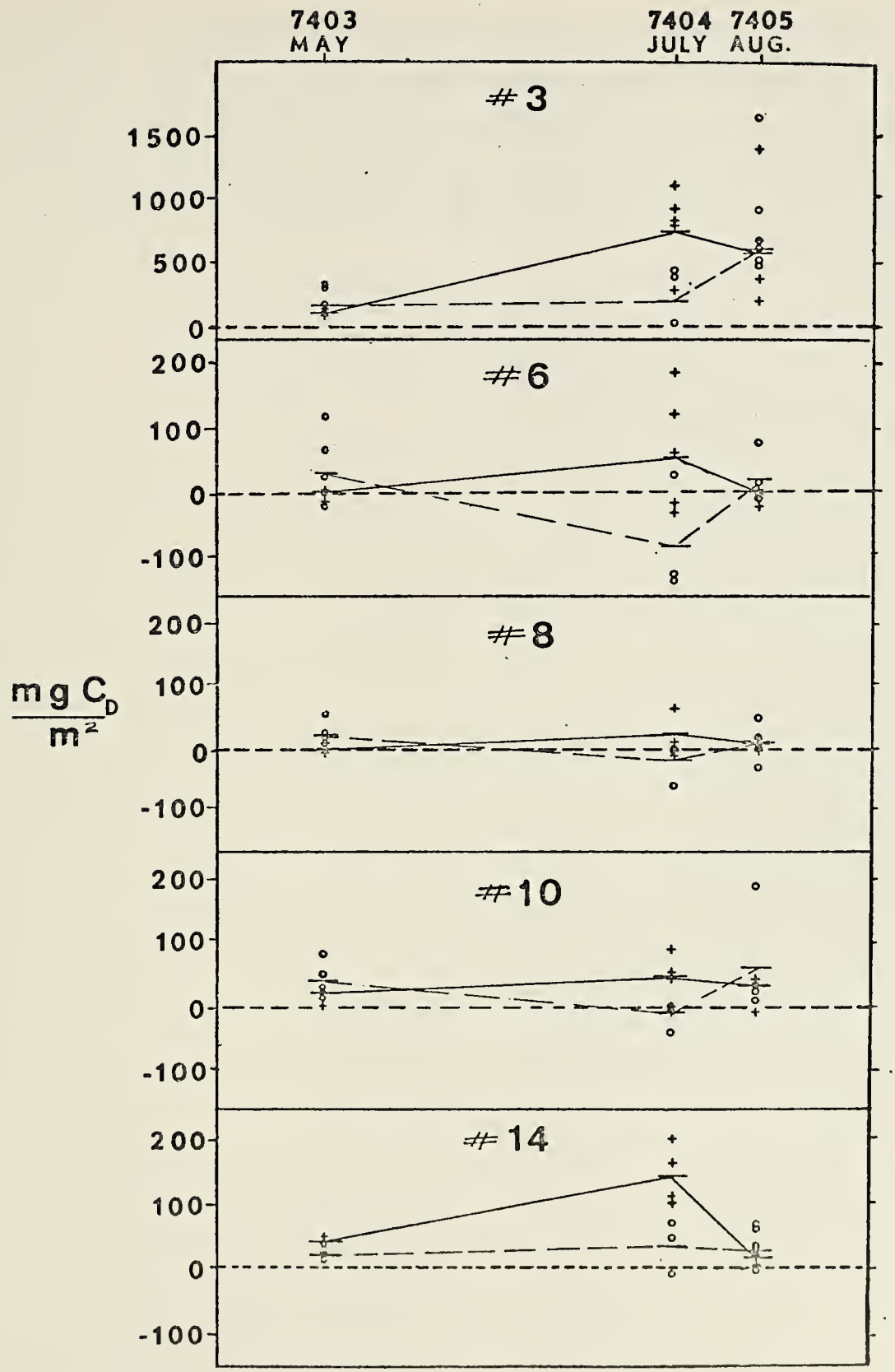


Figure 22(c). Dead carbon (C_D) per surface area. + = night station, o = day station; ___ = night, --- = day.

fractions. For example, in the most obvious case, the #3 net fraction (> 333 μm) which had no upper limit contained greater biomass than the lower fractions. The #6 net fraction (333-243 μm) also had a greater size range than #8, #10, and #14, and more biomass than did these fractions.

V. DISCUSSION AND CONCLUSIONS

A. LABORATORY WORK

It has been established that organic carbon analysis is a sensitive and reliable test of the biomass of marine organisms, *i.e.*, zooplankton. The use of the LECO Carbon Analyzer, adapted and used for all total carbon analyses, demonstrated a rapid (70 seconds), consistent, and accurate measurement of carbon in benzoic acid (a non-protein) and casein (a protein), the two chemical standards, in samples of less than 71 mg. Furthermore, the comparison of the two standard curves in Figures 14(b) and 15(b) of these organic substances reveals a very close relationship of DVM to carbon in the two different standards. If a benzoic acid or casein sample were combusted, therefore, the use of either standard curve to convert the DVM reading would yield the same measurement of carbon. There seems to be no interference of gaseous combustion products, *i.e.*, oxides of nitrogen, with the thermal conductivity cell of the LECO Carbon Analyzer as demonstrated by the similarity of the two standard curves and the infrared analyses. Thus, it was assumed for further carbon analyses that the measurement of carbon in zooplankton could be based on the "standard curve" for benzoic acid and casein, *i.e.*, direct conversions of DVM readings to zooplankton carbon could be made.

All DVM conversions to carbon in all but the standardization experiments were actually based on combined standard curve for benzoic acid and casein. The equation of the composite graph of DVM against weight of benzoic acid for three runs was found to be $Y = 0.0101 + 0.0465507\ X$ (Figure 14(a)). For the range of plankton samples in this study, ($\sim$5-20 mg)

the above equation was approximated by Y = 0.04656 X for computational purposes. The resulting standard curve, using this equation, a 10 mg sample of benzoic acid, and its carbon content, was Y = 0.06763 X. This equation was manipulated to yield X = 14.79 Y, where Y is the DVM reading and X is the carbon in mg. The equation of the composite graph of DVM against weight of casein for three runs was determined to be Y = 0.0073 + 0.03592. As before, for the sample range of interest, this equation was approximated by the following form: Y = 0.03592 X. The conversion of this equation to that of the standard curve, using a 10 mg casein sample and its known carbon content, resulted in the equation: Y = 0.06761 X. Manipulating this casein standard curve equation lead to the same result determined by the benzoic acid standard curve equation: X = 14.79 Y, where Y is the DVM reading and X is carbon in mg. Thus, measurements of carbon (mg) were obtained on the basis of multiplying the DVM value times 14.79. These approximations of slope to obtain a zero intercept could have lead to an error in carbon calculations of up to ±3% at high or low values by the standard curves. This error is still less than that found in other total carbon analyses which are usually greater than 5%.

The determination of the total or organic carbon content in *T. californicus* demonstrated a good measurement of carbon in a single copepod species. This mean carbon content of 38.6% of the freeze-dried weight agrees well with Curl's data on the carbon content in copepods (1962). Thus, it was shown that carbon analysis using the LECO Carbon Analyzer could be extended to freeze-dried plankton in determining total carbon (*i.e.*, the sum of living plus dead).

The examination of the ATP-C to total C ratio in *T. californicus* resulted in a mean ratio of 0.2634×10^{2} or 0.2634%, which could be used

to convert ATP to living carbon. The constancy of the determined ratio from the data in these experiments suggests that no nonliving particulate matter was present and that there was no difference in two different size groups. The two experiments were each conducted in a slightly different manner. The effect of filtration, tested in the first experiment, caused no significant changes in the ATP measurements of before and after filtration. Control analyses consisted of filtering the TRIZMA buffer solution by itself and were done to investigate the effect of the ATP extraction on ATP measurements of the solution passing through the filter, as well as carbon measurements of the freeze-dried filter. The result in both experiments was a small control correction, usually subtracted from the original measurements of ATP and carbon. Suspended carbon carried over in the ATP aliquot was corrected for in the first experiment, *i.e.*, 2/extraction volume x total C was added to total C. No corrections were made for dissolved non-ATP-C or possible losses on experimental apparatus. Corrections inherent in the ATP and carbon methods of measurement might have been necessary, but the constancy of the ATP-C to cell carbon ratio in the test organism suggests that consistent analysis procedures were used, and the ratio is accurate, assuming these corrections were insignificant. Separate sievings of the test organisms produced no significant differences in the ATP-C to total C ratio. No significant differences occurred between different size fractions. Two replicated size fractions (297-250 μm) were excluded from the combined data graph (their ratios were 0.24 and 0.35%) of ATP-C against total C in the determination of the mean ratio, since these fractions were not common to both experiments and may have contained detritus. Based on the assumption of live organisms in this experiment, the ATP-C to total C ratio examination provided a

measurement of the amount of living carbon (C_L) expressed as $R = \frac{ATP\text{-}C}{C_L}$. This relationship was later used in the form $C_L = \frac{1}{R}(ATP\text{-}C) \approx 380(ATP\text{-}C)$ to compute living carbon estimates in "net zooplankton" samples. These estimates of living biomass were based on the constancy of the ATP-C to total C ratio in *T. californicus* and its further application to all marine organisms caught by the nets.

B. FIELD STUDIES

No existing sampling net takes a representative sample of all types of zooplankton in any given area (Mullin, 1969). However, the in-line multiple net system utilized in this study was designed by Dr. Traganza in an attempt to separate trophic size groups by approximate size fractions which would include organisms which were predominantly herbivorous. The importance of herbivores in sound scattering models is a subject of another study (Traganza, 1974).

The sampling technique employed several hypotheses on the distribution of zooplankton in the ocean. For example, as illustrated by Blackburn, *et al* (1970) the amount of chlorophyll a below 150 m in the eastern tropical Pacific is negligible. Thus, the herbivores and phytoplankton were assumed to be in this upper layer of the ocean. The herbivorous plankton as grazers of the phytoplankton were generally assumed to be indigenous to this region or they migrate vertically to it from depth. A sampling depth of 200 m, which would include the euphotic zone, was thus chosen as the limiting depth of the vertical net tow. No correction was made for unintentional sampling at greater or lesser depths. Appendix D gives the depths of the tows.

The zooplankton populations were assumed not to be uniformly or randomly distributed, but rather in "patches" (Margelef, 1967) which are

strongly dependent on space and time manifestations of an organization related to hydrographic distributions such as eddies, areas of strong vertical mixing, upwelling domes, internal waves, Langmuir circulation, convergences and divergences, etc. (Traganza and Stewart, 1973). These "patches" were assumed to be on the order of miles or tens of miles (Cushing and Tungate, 1963). The practical approach used to consider these "patches" was to study a volume or water mass defined by coordinates relative to a drogue. Each cruise began from nearly the same geographic location. The use of the drogue to mark the water mass eliminated horizontal advectional effects and resulted in the station positions being taken relative to the drogue. The X-shaped station pattern was based on an optimum search technique which was used to minimize the zooplankton patchiness problem and locate maximum concentration of zooplankton in a search area (Traganza, 1974).

Estimates of total, living, and dead zooplankton biomass by carbon analysis and ATP measurements demonstrated a definite seasonal trend over the period of the three cruises. Explanation of these results is beyond the scope and intent of this thesis. However, it is clear that the methodology developed in this study is a rapid (70 seconds), precise (±3%) and accurate (±3%) measurement of zooplankton biomass carbon.

If such estimates of zooplankton biomass by carbon analysis are combined with biomolecular characteristics of zooplankton, *e.g.*, ATP, and related to chemical properties of the environment, predictive sound scattering models of the ocean may be feasible.

VI. RECOMMENDATIONS

After reviewing carbon research and reflecting on the experiences and knowledge gained in this thesis, the author makes the following recommendations:

(1) Further verification of the derived standard curves of the LECO Carbon Analyzer should be done with other standards in future studies which use them directly for carbon calculations in zooplankton.

(2) A sieve column for carbon samples, which would use stainless steel bolting cloth screens for concentration of the sample (these steel bolting cloth screens would fit into a crucible for direct carbon analysis after freeze-drying) should be tried.

(3) A more efficient commercial freeze-drier (2-3 hour drying time) is needed.

(4) Carbon analyses should be attempted at sea with the LECO Carbon Analyzer combined with one-week or longer cruises to make dirunal and several day studies to allow duplicate sampling.

(5) Acoustic measurements should be made in the same region to test the application of zooplankton biomass estimates to sound scattering.

APPENDIX A - VOLUME REVERBERATION THEORY

"The scattering of sound by biological populations in the upper layers of the ocean can place practical limits on the operation of low frequency (2 - 20 kHz) sonar" (Traganza and Stewart, 1973). The argument is outlined quantitatively from Batzler, Vent, and Davis (1968) as follows.

The echo level, EL, depends on the target strength, TS; the source level, SL; and a logarithmic function of the depth of the water column, H, such that

$$EL = SL + TS - 2H.$$

The volume reverberation level, RL_V, the most variable and unpredictable of the reverberation sources, depends on the source level, SL; the area insonified, A; the integrated water column scattering strength, S_V; and a logarithmic function of the depth, H, such that

$$RL_V = SL + 10 \log A + S_V - 2H$$

where

$$A = r\phi \times c\tau/2 \times \sec \Theta$$

c = speed of sound in seawater, τ = pulse duration, ϕ = beam width, Θ = transducer tilt, and r = horizontal range to the target. The integrated water column scattering strength, S_V, is defined as

$$S_V = 10 \log \int_{z_1}^{z_2} s_V(z)dz$$

where $s_V(z)$ is the scattering strength of any segment of the layer lying between depths z_1 and z_2.

S_v is dependent on the type and density of scatterers that give rise to reverberation and represents the amount of reverberation by all scatterers in a water column one square meter in area to a given depth.

APPENDIX B - THERMAL CONDUCTIVITY

The ability to detect almost any organic or inorganic compound has made the thermal conductivity detector reliable and widely recognised. Thermal conductivity is a transport phenomenon in that it is a transfer of kinetic energy due to a temperature gradient.

The principle determining the operation of the T/C detector is a difference in thermal conductivity of a carrier gas and a carrier gas-sample mixture. The heated filaments are cooled to a degree by a carrier gas stream and assume a definite resistance on one side of a four element cell. On the opposite side, the mixture of sample and carrier gas cools the filaments at a different rate. The resulting change in temperature causes a change in resistance and an unbalance is the change in composition of gas. Helium and hydrogen are commonly used for carrier gases because of their high thermal conductivity. Oxygen, the carrier gas used by the LECO Carbon Analyzer, also has a fairly high thermal conductivity of 6.35 cal $sec^{-1}cm^{-1}deg^{-1}$ at $27^{o}C$.

APPENDIX C - CALIBRATION INSTRUCTIONS

(After LECO Instruction Manual, 1974)

Listed below are the basic steps which one would take prior to analysis of samples using the LECO Carbon Analyzer after completion of the electronic checkout:

1. Turn on the FILAMENT switch of the induction furnace; after one minute, turn on the HIGH VOLTAGE switch of the induction furnace.
2. Open and close the leading tray of the induction furnace.
3. Turn the BLANK switch on the determinator OFF if it is on.
4. If the DVM HOLD switch is not glowing, depress it to turn it on.
5. Turn the FUNCTION SELECT switch to the OPERATE position.
6. Depress the ANALYZE switch to initiate the timing sequence. The switch will glow green before starting and will glow white at the time it is depressed and throughout the timing sequence. The oxygen should start flowing at this time also.
7. Set the oxygen flow through the purifying train to 1.5 liters per minute during the first 20 seconds after depressing the ANALYZE switch.
8. When the DVM HOLD and ANALYZE switches stop glowing, signifying an end to the 70 second timing sequence, open and close the furnace, but do not load a crucible.
9. Depress the ANALYZE switch to initiate a bridge balance determination. The determinator will go through the timing sequence again.
10. When the ANALYZE light stops glowing, the reading on the DVM should be 0.000 ± 0.002. If not, adjust the BRIDGE BALANCE FINE and/or COARSE controls located behind the swing-out access panel to bring the reading within specifications.

11. Repeat steps 8 through 10 with the DVM HOLD glowing for verification of bridge balance zero. Repeat if necessary.

12. Place a scoop of LECO iron chip accelerator and a scoop of LECO copper metal accelerator in a crucible.

13. Open the loading tray and place the loaded crucible on the ceramic pedestal. Close the loading tray and swing the counterweight arm against the POWER button of the induction furnace.

14. Depress the ANALYZE and DVM HOLD switches to start the timing sequence. The oxygen flow will not go to zero during pre-burn as before. The induction furnace plate current should go to between 400 to 500 milliamps before the red light of the induction furnace stops glowing.

15. The reading on the DVM will give an indication of the percentage content of argon in the oxygen tank. Depress the BLANK switch to turn it on and depress the DVM HOLD to turn it off. Adjust the BLANK control to bring the DVM reading to approximately 0.000% C. This is a rough setting of the BLANK.

16. Repeat steps 12 through 16 with the BLANK switch on for a verification of the blank adjustment.

17. Repeat steps 8 through 10 with the DVM HOLD glowing for verification.

18. Repeat steps 12 through 16 with the BLANK switch on for a final "blank" adjustment.

The blanking process can be further amplified by finer calibration with LECO steel rings. These instructions are given in the LECO Instruction Manual. This amplifying procedure was not used due to the time involved with further calibration and the number of samples required to complete the steel ring calibration. The very slight increase in accuracy due to this calibration was not worthwhile for the scope of the

carbon analyses reported in this work. Thus, steps 1 through 18 comprise "the blanking process" completed before each analysis.

CRUISE Tg 7403 30-31 May '74 GENERAL PURPOSE CODING FORM — Corrected values

Subtract 9 hrs to get LMT

0 = ZERO, 1 = ONE, 2 = TWO, 3 = ALPHA O, I = ALPHA I, Z = ALPHA Z

ST	TIME (GMT)	DEPTH	#3 mgC/m²	#3 C_D mg/m²	#6 mgC/m²	#6 C_D mg/m²	#8 mgC/m²	#8 C_D mg/m²	#10 mgC/m²	#10 C_D mg/m²	#14 mgC/m²	#14 C_D mg/m²	Σ mgC/m²	Σ C_D mg/m²	Σ C_a mg/m²	Code 3 6 8 10 14
01	0425	201	275.	187.31	33.66	4.80	14.88	1.870	16.34	10.04	24.88	22.64	364.9	117.1	247.8	
02	0810	183	212.	239.28	77.32	15.12	16.83	4.427	43.66	32.85	56.59	48.85	406.6	140.5	266.1	I
03	1525	201	76.	103.047	98.29	29.45	54.88	26.47	50.24	35.68	27.56	21.19	307.1	115.8	191.3	
04	1825	207	231.	0291.9	207.6	123.5	96.59	56.76	116.1	85.26	50.49	31.64	701.8	499.1	202.7	
05	2030	201	157.	873.22	91.95	4.635	60.24	11.65	47.32	16.03	17.80	6.762	375.1	112.3	262.8	
06	2300	189	205.	970.57	58.29	-19.24	8.548	2.265	79.27	32.84	24.39	5.463	416.4	91.94	324.5	*
07	0100	201	378.	8233.8	167.6	71.30	43.42	20.13	74.88	52.12	282.4	?	947.1	377.4	569.7	*

N. night

? did not get data

I. Infrared Sample

* Net torn

CRUISE Tg 7403 30-31 May '74 GENERAL PURPOSE CODING FORM Carbon values uncorrected for controls.

Subtract 9 hrs to get LMT

0 = ZERO
Ø = ALPHA O

1 = ONE
I = ALPHA I

2 = TWO
Z = ALPHA Z

(1st net system)

ST	TIME (GMT)	DEPTH	DVM	mgC	#3 DVM	#3 mgC	#6 DVM	#6 mgC	#8 DVM	#8 mgC	#10 DVM	#10 mgC	#14 DVM	#14 mgC	Σ	Σ mgC	Code
01	0125	201			0.787	11.63	0.117	1.73	0.065	0.96	0.069	1.02	0.093	1.37	16	6.71	
02	0810	183	+.035	0.52	0.612	9.05	0.238	3.52	0.082	1.21	0.156	2.31	0.192	2.84	18	8.93	
03	1525	201			0.235	3.47	0.296	4.38	0.176	2.60	0.163	2.41	0.100	1.48	14	4.34	
04	1825	207	+.029	0.43	0.670	9.90	0.605	8.94	0.297	4.39	0.351	5.19	0.169	2.50	30	0.92	
05	2030	201	+.013	0.19	0.447	6.66	0.268	3.96	0.180	2.66	0.144	2.13	0.062	0.92	16	6.33	
06	2300	189	+.022	0.33	0.593	8.77	0.184	2.72	0.157	2.32	0.242	3.58	0.090	1.33	18	8.72	*
07	0100	201	+.020	0.30	1.071	15.83	0.485	7.17	0.141	2.08	0.228	3.37	0.804	11.88	40	0.33	*

average control = +.024

* Net torn

CRUISE Tg 7403 30-31 May '74 GENERAL PURPOSE CODING FORM Carbon values uncorrected for controls.

Subtract 9 hrs to get LMT

0 = ZERO
Ø = ALPHA O
1 = ONE
I = ALPHA I
2 = TWO
Z = ALPHA Z
(1st not system)

ST	TIME (GMT)	LATITUDE ° ' N	LONGITUDE ° ' W	#3 mgC	#3 mgM	#6 mgC	#6 mgM	#8 mgC	#8 mgM	#10 mgC	#10 mgM	#14 mgC	#14 mgM	ΣmgC	ΣmgM
1	0425	36-46.9	122-00.8	11.63	?	1.73	5.0	0.96	1.5	1.02	3.0	1.37	2.7	16.71	?
2	0810	36-44.2	122-03.7	9.05	13.4	3.52	5.8	1.21	?	2.31	5.2	2.84	6.8	18.93	?
3	1525	36-46.9	122-00.8	3.47	20.7	4.38	16.0	2.60	11.5	2.41	8.3	1.48	7.5	14.34	83.2
4	1825	36-44.0	122-09.2	9.90	25.2	8.94	21.8	4.39	13.9	5.19	19.1	2.50	14.3	30.92	94.3
5	2030	36-47.5	122-06.0	6.66	23.1	3.96	13.2	2.66	12.8	2.13	12.4	0.92	9.4	16.33	70.9
6	2300	36-46.3	122-04.2	8.77	34.9	2.72	10.9	2.32	10.2	3.58	14.2	1.33	10.1	18.72	80.3
7	0100	36-46.9	122-00.8	15.83	46.3	7.17	26.0	2.08	14.5	3.37	17.4	11.88	42.8	40.33	147.0

M = mass
C = Carbon
? did not get data

CRUISE Tg 7404 24 July '74 GENERAL PURPOSE CODING FORM

Corrected values

Subtract 9 hrs. to get LMT ↓ | O = ZERO Ø = ALPHA O | I = ONE I = ALPHA I | Z = TWO Z = ALPHA Z

	ST.	TIME (GMT)	DEPTH	#3 mgC/m²	#3 C_D mg/m²	#6 mgC/m²	#6 C_D mg/m²	#8 mgC/m²	#8 C_D mg/m²	#10 mgC/m²	#10 C_D mg/m²	#14 mgC/m²	#14 C_D mg/m²	Σ mgC/m²	Σ C_D mg/m²	ΣC_4 mg/m²	Code 3 6 8 10 A
	01	0215	204	1637.	?	106.1	?	35.12	?	110.2	?	117.6	?	2006.	?	?	
N	02	0610	201	1481.	1049.	205.1	198.0	112.4	73.14	138.8	100.8	186.8	164.3	2124.	1585.	539.0	
N	03	0815	250	914.9	779.5	218.3	134.6	70.24	20.48	110.2	55.05	145.1	100.1	1459.	1090.	369.0	
N	04	0950	203	1446.	862.6	70.24	-21.8	21.10	-3.97	117.8	65.76	252.7	203.5	1908.	1106.	802.0	X
N	05	1145	305	390.0	230.4	191.0	74.02	32.68	8.746	23.41	3.762	159.3	113.6	796.4	430.5	365.9	
N	06	1330	213	1361.	739.3	82.93	-4.46	27.80	7.659	51.95	10.04	169.3	103.8	1693.	856.4	836.6	
	07	1925	201	862.4	296.1	131.	232.24	17.32	3.779	27.80	5.118	125.4	62.17	1164.	399.4	764.6	
	08	2140	201	584.4	-77.1	99.02	-125.	7.244	-2.24	56.10	-2.39	42.93	-19.4	854.9	-226.	1081	
	09	2310	202	760.	234	7.1	123.4	-134.	14.39	-57.7	74.39	-38.9	116.8	39.04	1089.	155.5	933.5

No night

? did not get data

X - part of sample lost

111

CRUISE Tg 7404 24 July '74 GENERAL PURPOSE CODING FORM Carbon values uncorrected for controls.

Subtract 9hrs to get LMT

O = ZERO I = ONE 2 = TWO

Q = ALPHA O I = ALPHA I Z = ALPHA Z

ST	TIME (GMT)	DEPTH	CONTROL DVM	CONTROL mgC	#3 DVM	#3 mgC	#6 DVM	#6 mgC	#8 DVM	#8 mgC	#10 DVM	#10 mgC	#14 DVM	#14 mgC	Σ	Σ mgC	Code 36 8 10 14
01	0215	204			4.548	67.32	0.309	4.57	0.112	1.66	0.321	4.74	0.341	5.04	83	.33	
02	0610	201			4.123	60.94	0.584	8.63	0.327	4.83	0.400	5.91	0.533	7.88	88	.19	
03	0815	250	+.035	0.52	2.573	38.03	0.641	9.47	0.230	3.40	0.341	5.04	0.438	6.47	62	.41	
04	0950	203	+.025	0.37	4.037	59.67	0.220	3.25	0.088	1.30	0.352	5.20	0.726	10.73	80	.15	x
05	1145	305	+.010	0.15	1.092	16.14	0.540	7.98	0.101	1.49	0.075	1.11	0.452	6.68	33	.40	
06	1330	213	+.003	0.04	3.779	55.86	0.233	3.44	0.080	1.18	0.147	2.17	0.472	6.98	69	.63	
07	1925	201	-.002	-0.03	2.394	35.39	0.366	5.41	0.050	0.74	0.079	1.17	0.350	5.17	47	.88	
08	2140	201	+.016	0.24	1.637	24.20	0.291	4.30	0.217	3.21	0.172	2.54	0.135	2.00	36	.25	
09	2310	202			2.124	31.39	0.357	5.28	0.055	0.81	0.221	3.27	0.339	5.01	45	.76	

average control = .015

X - part of sample lost

CRUISE Tg 7404 24 July '74 GENERAL PURPOSE CODING FORM Carbon values uncorrected for controls.

Subtract 9 hrs to get LMT O = ZERO I = ONE Z = TWO (2nd generation net system)

O = ALPHA O I = ALPHA I Z = ALPHA Z

St.	TIME (GMT)	LATITUDE ° ' N	LONGITUDE ° ' W	#3 mgC	#3 mgM	#6 mgC	#6 mgM	#8 mgC	#8 mgM	#10 mgC	#10 mgM	#14 mgC	#14 mgM	Σ mgC	Σ mgM
1	0215	36-41.8	122-06.0	67.22	?	4.57	19.5	1.66	13.8	4.74	23.1	5.04	18.0	83.33	?
2	0610	36-44.0	122-03.7	60.94	108.2	8.63	37.3	4.83	28.5	5.91	56.9	7.88	35.1	88.19	266.0
3	0815	36-44.8	122-07.0	38.03	62.3	9.47	28.4	3.40	8.5	5.04	14.3	6.47	19.5	62.41	133.0
4	0950	36-44.0	122-05.3	59.67	128.2	3.25	7.8	1.30	4.3	5.20	9.7	10.73	27.1	80.15	177.1
5	1145	36-42.8	122-07.1	16.14	26.7	7.98	14.2	1.49	3.6	1.11	3.9	6.68	21.6	33.40	70.0
6	1330	36-43.3	122-03.6	55.86	109.2	3.44	10.6	1.18	3.6	2.17	6.1	6.98	14.6	69.63	144.1
7	1925	36-47.3	122-03.1	35.39	95.9	5.41	13.8	0.74	2.6	1.17	3.6	5.17	14.6	47.88	130.5
8	2140	36-47.1	122-06.7	24.20	10.8	4.30	15.0	3.21	7.9	2.54	12.3	2.00	10.7	36.25	156.7
9	2310	36-46.2	122-04.8	31.39	56.1	5.28	13.5	0.81	4.0	3.27	8.2	5.01	14.5	45.76	96.3

M = mass C = carbon ? did not get data

CRUISE Tg 7405 6-7 Aug. '74 GENERAL PURPOSE CODING FORM — Corrected values

Subtract 9 hrs. to get LIGHT

0 = ZERO, Ø = ALPHA O; 1 = ONE, I = ALPHA I; 2 = TWO, Z = ALPHA Z

Code

	ST	TIME	DEPTH	#3 mgC/m²	#3 C_D mg/m²	#6 mgC/m²	#6 C_D mg/m²	#8 mgC/m²	#8 C_D mg/m²	#10 mgC/m²	#10 C_D mg/m²	#14 mgC/m²	#14 C_D mg/m²	Σ mgC/m²	Σ C_D mg/m²	Σ C_L mg/m²	3 6 8 10 14
	01	0235	189	640.5	385.9	86.34	83.68	52.93	51.26	190.7	190.3	66.34	60.73	1037.	771.9	265.1	
N	02	0655	159	518.0	151.1	73.90	16.63	36.09	24.90	77.80	55.61	43.66	19.38	749.5	267.6	481.9	
N	03	0830	201	1749.	1336.	99.76	-11.2	29.76	6.471	112.4	53.65	4.878	4.347	1996.	1389.	607.0	
N	04	0930	189	781.0	322.4	128.8	5.452	47.32	8.176	14.63	2.834	30.00	17.52	1002.	356.4	645.6	
	05	1835	201	1880.	1549.	119.0	-7.57	33.90	1.697	53.41	12.18	70.00	53.42	2156.	1609.	547.0	
	06	2015	201	940.7	589.9	69.27	-4.96	45.37	8.047	37.32	12.55	5.610	-15.2	1098.	590.3	507.7	
	07	2200	186	779.8	436.4	130.5	21.64	23.17	-28.2	?	?	26.59	12.56	960.1	442.4	517.7	?
	08	2315	201	1145.	829.8	84.15	3.397	32.93	20.41	58.78	26.31	34.39	26.42	1355.	906.3	448.7	
	09	0020	100	855.1	527.4	87.31	?	61.46	5.930	65.85	37.55	31.95	22.39	1102.	593.3	508.7	Q

N = night

Q - depth of bottom limited cast; ? did not get data

CRUISE Tg 7405 6-7 Aug '74 GENERAL PURPOSE CODING FORM Carbon values uncorrected for controls.

Subtract 9 hrs. to get LMT 0 = ZERO 1 = ONE 2 = TWO 0 = ALPHA O I = ALPHA I Z = ALPHA Z

ST	TIME (GMT)	DEPTH	CONTROL DVM	CONTROL mgC	#3 DVM	#3 mgC	#6 DVM	#6 mgC	#8 DVM	#8 mgC	#10 DVM	#10 mgC	#14 DVM	#14 mgC	Σ mgC	Code 3 6 8 10 14
01	0235	189	+.018	0.27	1.795	26.53	0.258	3.81	0.165	2.44	0.547	8.09	0.202	2.99	43.86	
02	0655	159	+.004	0.06	1.441	21.30	0.209	3.09	0.104	1.54	0.220	3.25	0.125	1.85	31.03	
03	0830	201	+.009	0.13	4.857	71.84	0.285	4.22	0.091	1.35	0.321	4.74	0.022	0.33	82.48	
04	0930	189	+.010	0.15	2.175	32.17	0.367	5.43	0.141	2.09	0.051	0.75	0.093	1.38	41.82	
05	1835	201	+.007	0.21	5.226	77.29	0.344	5.09	0.108	1.60	0.162	2.40	0.208	3.08	89.46	
06	2015	201	+.010	0.30	2.628	38.87	0.212	3.14	0.146	2.16	0.124	1.83	0.036	0.53	46.53	
07	2200	186	+.004	0.12	2.170	32.09	0.370	5.47	0.072	1.07	?	?	0.082	1.21	?	?
08	2315	201			3.184	47.09	0.242	3.58	0.100	1.48	0.172	2.54	0.104	1.54	56.23	
09	0020	100	+.013	0.38	2.396	35.44	0.268	3.96	0.196	2.90	0.208	3.08	0.114	1.69	47.07	

average control = +.009

? did not get data

CRUISE Tg 7405 6-7 Aug '74 GENERAL PURPOSE CODING FORM Carbon values uncorrected for controls.

Subtract 9 hrs. to get LMT 0 = ZERO Ø = ALPHA O 1 = ONE I = ALPHA I 2 = TWO Z = ALPHA Z

ST	TIME (GMT)	LATITUDE ° ' N	LONGITUDE ° ' W	#3 mg C	#3 mg M	#6 mg C	#6 mg M	#8 mg C	#8 mg M	#10 mg C	#10 mg M	#14 mg C	#14 mg M	Σ mg C	Σ mg M
1	0235	36-39.3	121-57.5	26.53	84.2	3.81	27.3	2.44	15.2	8.09	54.1	2.99	20.0	43.86	200.8
2	0655	36-42.3	122-03.3	21.30	76.7	3.09	12.8	1.54	9.8	3.25	14.5	1.85	8.6	31.03	122.4
3	0830	36-44.0	122-01.5	71.84	167.3	4.22	8.0	1.35	6.3	4.74	16.0	0.33	1.4	82.48	199.0
4	0930	36-43.8	122-04.8	32.17	63.0	5.43	12.3	2.09	4.7	0.75	0.8	1.38	3.5	41.82	84.3
5	1835	36-44.9	121-58.9	77.29	161.4	5.09	15.2	1.60	13.4	2.40	17.6	3.08	18.4	89.46	226.0
6	2015	36-44.9	122-02.2	38.87	80.0	3.14	11.0	2.16	15.4	1.83	7.2	0.53	10.0	46.53	123.6
7	2200	36-43.5	121-59.9	32.09	113.6	5.47	18.0	1.07	14.4	?	?	1.21	13.8	?	?
8	2315	36-41.8	122-01.1	47.09	165.8	3.58	10.2	1.48	11.0	2.54	11.4	1.54	7.2	56.23	205.6
9	0020	36-42.3	121-57.2	35.44	162.4	3.96	16.0	2.90	20.4	3.08	23.6	1.69	8.8	47.07	231.2

M = mass C = carbon ? did not get data

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